MICROCIRCUITS

THE INTERFACE BETWEEN NEURONS AND GLOBAL BRAIN FUNCTION

EDITED BY S. GRILLNER AND A. M. GRAYBIEL

DAHLEM WORKSHOP REPORTS

VISIT…

Microcircuits

The Interface between Neurons and Global Brain Function

Goals for this Dahlem Workshop:

- To discuss how microcircuits could act as elementary processing units to bridge single cells to systems and behavior,
- To compare the intrinsic function of microcircuits with their ion channel subtypes, connectivity, and receptors to arrive at an understanding of the design principles and their function.

Report of the 93rd Dahlem Workshop on
Microcircuits: The Interface between Neurons and Global Brain Function
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Microcircuits

The Interface between Neurons and Global Brain Function

Edited by

S. Grillner and A. M. Graybiel

Program Advisory Committee:

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J.-P. Changeux, A. M. Graybiel, T. Isa, H. Markram,
R. Menzel, and H.-J. Pflüger

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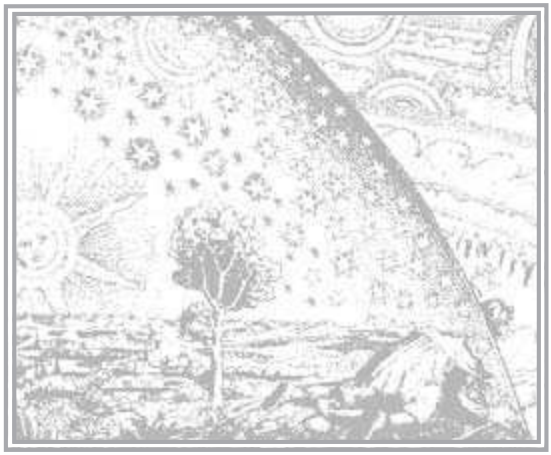
Dahlem Workshops



History

During the last half of the twentieth century, specialization in science greatly increased in response to advances achieved in technology and methodology. This trend, although positive in many respects, created barriers between disciplines, which could have inhibited progress if left unchecked. Understanding the concepts and methodologies of related disciplines became a necessity. Reuniting the disciplines to obtain a broader view of an issue became imperative, for problems rarely fall conveniently into the purview of a single scientific area. Interdisciplinary communication and innovative problem-solving within a conducive environment were perceived as integral yet lacking to this process.

In 1971, an initiative to create such an environment began within Germany's scientific community. In discussions between the *Deutsche Forschungsgemeinschaft* (German Science Foundation) and the *Stifterverband für die Deutsche Wissenschaft* (Association for the Promotion of Science Research in Germany), researchers were consulted to compare the needs of the scientific community with existing approaches. It became apparent that something new was required: an approach that began with state-of-the-art knowledge and proceeded onward to challenge the boundaries of current understanding; a form truly interdisciplinary in its problem-solving approach.

As a result, the *Stifterverband* established *Dahlem Konferenzen* (the Dahlem Workshops) in cooperation with the *Deutsche Forschungsgemeinschaft* in 1974. Silke Bernhard, formerly associated with the Schering Symposia, was

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Figure adapted from *L'Atmosphère: Météorologie Populaire*, Camille Flammarion. Paris: Librairie Hachette et Cie., 1888.

engaged to lead the conference team and was instrumental in implementing this unique approach.

The Dahlem Workshops are named after a district of Berlin known for its strong historic connections to science. In the early 1900s, Dahlem was the seat of the Kaiser Wilhelm Institutes where, for example, Albert Einstein, Lise Meitner, Fritz Haber, and Otto Hahn conducted research. Today the district is home to several Max Planck Institutes, the *Freie Universität Berlin*, the *Wissenschaftskolleg*, and the Konrad Zuse Center.

In its formative years, the Dahlem Workshops evolved in response to the needs of science. They soon became firmly embedded within the international scientific community and were recognized as an indispensable tool for advancement in research. To secure its long-term institutional stability, *Dahlem Konferenzen* was integrated into the *Freie Universität Berlin* in 1990.

Aim

The aim of the Dahlem Workshops is to promote an international, interdisciplinary exchange of scientific information and ideas, to stimulate international cooperation in research, and to develop and test new models conducive to more effective communication between scientists.

Concept

The Dahlem Workshops were conceived to be more than just another a conference venue. Anchored within the philosophy of scientific enquiry, the Dahlem Workshops represent an independently driven quest for knowledge: one created, nurtured, and carefully guided by representatives of the scientific community itself. Each Dahlem Workshop is an interdisciplinary communication process aimed at expanding the boundaries of current knowledge. This dynamic process, which spans more than two years, gives researchers the opportunity to address problems that are of high-priority interest, in an effort to identify gaps in knowledge, to pose questions aimed at directing future inquiry, and to suggest innovative ways of approaching controversial issues. The overall goal is not necessarily to exact consensus but to search for new perspectives, for these will help direct the international research agenda.

Governance

The Dahlem Workshops are guided by a Scientific Advisory Board, composed of representatives from the international scientific community. The board is responsible for the scientific content and future directions of the Dahlem Workshops and meets biannually to review and approve all workshop proposals.

Workshop Topics

Workshop topics are problem-oriented, interdisciplinary by nature, of high-priority interest to the disciplines involved, and timely to the advancement of science. Scientists who submit workshop proposals, and chair the workshops, are internationally recognized experts active in the field.

Program Advisory Committee

Once a proposal has been approved, a Program Advisory Committee is formed for each workshop. Composed of 6–7 scientists representing the various scientific disciplines involved, the committee meets approximately one year before the Dahlem Workshop to develop the scientific program of the meeting. The committee selects the invitees, determines the topics that will be covered by the pre-workshop papers, and assigns each participant a specific role. Participants are invited on the basis of their international scientific reputation alone. The integration of young German scientists is promoted through special invitations.

Dahlem Workshop Model

A Dahlem Workshop can best be envisioned as a week-long intellectual retreat. Participation is strictly limited to forty participants to optimize the interaction and communication process.

Participants work in four interdisciplinary discussion groups, each organized around one of four key questions. There are no lectures or formal presentations at a Dahlem Workshop. Instead, concentrated discussion—within and between groups—is the means by which maximum communication is achieved.

To enable such an exchange, participants must come prepared to the workshop. This is facilitated through a carefully coordinated pre-workshop dialog: Discussion themes are presented through "background papers," which review a particular aspect of the group's topic and introduce controversies as well as unresolved problem areas for discussion. These papers are circulated in advance, and everyone is requested to submit comments and questions, which are then compiled and distributed. By the time everyone arrives in Berlin, issues have been presented, questions have been raised, and the Dahlem Workshop is ready to begin.

The discussion unfolds in moderated sessions as well as during informal times of interaction. Cross-fertilization between groups is both stressed and encouraged. By the end of the week, through a collective effort directed that is directed by a rapporteur, each group has prepared a draft report of the ideas, opinions, and contentious issues raised by the group. Directions for future research are highlighted, as are problem areas still in need of resolution. The results of the draft reports are discussed in a plenary session on the final day and colleagues from the Berlin–Brandenburg area are invited to participate.

Dahlem Workshop Reports

After the workshop, attention is directed toward the necessity of communicating the perspectives and ideas gained to a wider audience. A two-tier review process guides the revision of the background papers, and discussion continues to finalize the group reports. The chapters are carefully edited to highlight the perspectives, controversies, gaps in knowledge, and future research directions.

The publication of the workshop results in book form completes the process of a Dahlem Workshop, as it turns over the insights gained to the broad scientific community for consideration and implementation. Each volume in the Dahlem Workshop Report series contains the revised background papers and group reports as well as an introduction to the workshop themes. The series is published in partnership with The MIT Press.

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Cortex, spines

1

Introduction

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During the last decade, progress in neuroscience has been phenomenal. On the one hand, at the gene–molecular–cellular level, we now have details of the intrinsic function of individual molecules such as ion channels, receptors, and the molecular machinery of a single synapse. This knowledge, derived from the cloning of several genomes, including our own, has taught us that there are limited genomic differences among vertebrates, and that many of our genes are shared with worms, flies, and yeast cells. Yet even with an understanding of the function of every single cell in the nervous system, we would still not comprehend the function of the nervous system itself without additional detailed knowledge of how the billions of nerve cells interact to form networks, as well as knowledge about the mechanisms by which they are called into action and perform their tasks.

New brain imaging techniques, such as PET and functional MRI, have contributed importantly by telling us where things happen in the brain, be it the formation of memories, the execution of different cognitive tasks, or the performance of motor activities. These techniques have brought cognitive psychology into the brain sciences, as it has been possible to document which regions are involved when solving any of the intelligent questions posed by an investigator to the experimental subjects or patients. As important as this has been, the resolution of these techniques is still limited to at best thousands of nerve cells. The techniques can thus only inform us about where things occur in the brain, and sometimes when, but not about the neuronal mechanisms that underlie a particular function, that is, the cellular bases of behavior.

Fortunately, the nervous system, with its 10^{11}–10^{12} nerve cells, is divided into functional groupings—specialized neuronal networks that subserve particular functions, such as feature detectors in a sensory system, cortical columns, and the networks underlying different patterns of motor behavior. At this Dahlem Workshop, we referred to such functional groupings as microcircuits: well-defined, fairly small entities of nerve cells, and we focused on the intrinsic function of these microcircuits.

For contemporary neuroscience, this level of analysis poses a demanding challenge. The aim is to understand the intrinsic function of such microcircuits and to bridge from the level of the gene and molecule to global brain function, which means that we need to bridge from:

gene/molecule → cell → synapse → network →
neural subsystem → behavior/cognition.

If we are to reach an understanding of the function of a given gene or molecule, it is not sufficient to show that it affects a certain behavior or produces a particular malfunction. There is no way around the fact that to comprehend, we must actually get a good grasp of the intervening steps between molecule and global brain function. The most difficult and challenging level is that of understanding the cellular basis of a microcircuit—the dynamic mechanism involved in a functional network. As a minimal requirement, we must know which cells take part, what their membrane properties are, and how they talk to each other via synapses.

In the tradition of the Dahlem Workshops, we subdivided the theme of microcircuits into four fundamentally different types of well-defined microcircuits and looked for common cellular mechanisms: microcircuits in the motor system (Group 1), microcircuits in the striatum (Group 2), olfactory microcircuits (Group 3), and neocortical microcircuits (Group 4).

The microcircuits in the motor system are best defined in terms of their function. We addressed three vertebrate circuits that are now comparatively well understood: those coordinating locomotion, respiration, and the saccadic eye movements. These represent three neuronal networks with different functions that operate on different timescales. In some vertebrate species, these networks are understood at a level at which one can link from a gene product to intrinsic network function and therefore to motor behavior. In most instances, however, only the general properties of the networks have been defined, such as the mechanisms releasing a given motor pattern (for example, a rapid eye movement triggered from the superior colliculus of the mesencephalon). A common principle emerged from the discussion in that the core of each pattern of motor activity appears to depend on excitatory glutamatergic neurons that interact and generate burst activity. Membrane properties with specific ion channel subtypes serve to terminate the burst activity. This core unit burst-generating mechanism was a common theme that emerged from the group discussions. Inhibitory mechanisms contribute to patterns of interaction among the bursting microcircuits, or even serve to release them, as is true for saccadic eye movements.

The microcircuits in the striatum, which is the largest input station of the basal ganglia, are important for both motor function and cognition. Malfunction of the striatum can lead to a decreased ability to generate well-coordinated movements, as in Parkinson's disease, or conversely elicit involuntary movements, as in Huntington's disease. The basal ganglia are also implicated in

neuropsychiatric disorders. The goal of this group was to try to identify a core striatal microcircuit and to delineate striatal microcircuitry that most likely is part of the decision-making functions of the striatum contributing to the dynamic selection of different patterns of behavior. The detailed study of interactions among the different classes of medium spiny neurons with inputs from the cortex, the thalamus, and dopamine-containing neurons, as well as inputs from local interneurons, have clarified issues central to the cellular interactions in striatal microcircuits and their mechanisms of synaptic plasticity. Of importance in bridging from the molecular level to the circuit level is an understanding of the membrane properties of the different cell types. For instance, output neurons of the striatum have a high threshold for spike firing, so that they can serve as a filter in the pathway from the cerebral cortex through the basal ganglia.

The surprising finding in the early 1990s, that olfaction is coded by a large gene family of a thousand genes in mice, has radically changed our view of the function of this sensory system. In each of the olfactory receptor cells, only one of these genes is expressed, so they are functionally specific. Olfactory cells of a similar type, in turn, project to common target microcircuits in the olfactory bulb called glomeruli. All olfactory receptor cells that express a given gene project to the same glomerular subtype, and from these glomeruli, mitral cells project farther into the brain. The goal of the olfactory system group was to discuss the microcircuitry within the glomerulus and the circuits connecting glomeruli. The neural organization with glomeruli is similar in invertebrates, such as the bee, and all vertebrates so far studied. The significance of such a conserved organization was highlighted. The structure of the glomeruli themselves, with their surrounding membrane and comparatively few glial cells, was suggested to serve as an amplifier for incoming olfactory signals because the presumed transmitter, glutamate, could stay around for a prolonged period of time in the absence of glial clearance. The dynamic processing of mitral cells with interacting GABAergic cells generates synchronous oscillations, which have potentially important signaling functions.

It is generally assumed that cognitive functions rely heavily on processing in the neocortex. To understand the underlying mechanisms, it is not sufficient to use brain imaging and the recordings of a single or multiple nerve cells during behavior. We also need to understand how the inhibitory and excitatory neurons in the neocortex interact, both locally in the range of a few hundred microns and via long-distance interactions with other parts of neocortex and with other structures (e.g., thalamus, striatum, and brainstem centers). The concept of functional modules in the cortex in the form of cortical columns has been a center of interest for several decades. The conclusion of this working group was, however, that many parts of the neocortex investigated thus far contain what appear to be a continuous sheet of different subclasses of interneurons and output neurons, rather than a mosaic of pre-formed cortical columns. Inputs from different structures, including the thalamus and the surrounding neocortex, appear to set

the pattern of activity in cortical microcircuits, forming dynamically changing subunits. Functional modularity may account for the recordings of distinct columnar activity, such as those defined by Vernon Mountcastle many decades ago.

The scientists invited to this meeting provided a new, stimulating mix of research interests. The interaction within the working groups was productive, and the exchanges across different neural systems provided interesting parallels. For instance, the excitatory core of the motor microcircuits is organized in a similar way to that of neocortical microcircuits.

The format of the Dahlem Workshop was particularly well suited for this microcircuit field, as it allowed more time for interaction than available at a conventional specialized symposium. We express our gratitude to the Scientific Advisory Board of the "Dahlem Konferenzen der Freien Universität Berlin" for their support of this meeting, including the dedicated contribution by Julia Lupp and her team, and to the Deutsche Forschungsgemeinschaft for its financial support.

2

Microcircuit of the Superior Colliculus

A Neuronal Machine that Determines Timing and Endpoint of Saccadic Eye Movements

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ABSTRACT

The superior colliculus (SC) plays a key role in controlling the timing and endpoints of saccadic eye movements. Although the activity of collicular neurons in awake animals and anatomical connection with other CNS structures have been intensively studied, information processing in the local circuits of the SC is only poorly understood.

During the last decade, however, whole-cell patch clamp recording techniques have been introduced in slice preparation in combination with intracellular staining techniques as a powerful tool for studying the intrinsic circuitry and biophysical properties of neurons in the local circuits of the SC. Ongoing *in vitro* studies are giving us definitive descriptions on the operation of the local circuits, which could never be obtained by conventional techniques.

In this chapter, we summarize the current status of *in vitro* approaches to the SC functions and speculate about how research that combines the *in vitro* and *in vivo* preparations will facilitate further understanding of the oculomotor control.

INTRODUCTION

Some neuroscientists interested in the neural control of movement study rhythmical behaviors, such as locomotion, respiration, or mastication. Others

examine more episodic behaviors, such as reaching movements of the arm or orienting movements of the eyes and head. Because the neural circuits involved in rhythmical behavior often continue to generate cyclical patterns of activity in isolated preparations (dish, slice, or slab), detailed descriptions of the biophysical and pharmacological properties of neurons and circuits are possible. These descriptions extend to studies of the effects of manipulating different subtypes of ion channels and transmitters or second-messenger systems on the behavior of the circuit (Grillner 2003). Some model systems have identified cells (the same cell can be found in different members of the species), and the anatomical connections of local networks of cells are completely described. The mechanisms by which neuromodulators alter the properties of a "hard-wired" circuit have been the focus of much work on the stomatogastric ganglion (Katz and Harris-Warrick 1999; Marder and Thirumalai 2002). Analytical models of circuit and cell behavior can be combined with experiments by using the dynamic clamp technique (Marder and Abbott 1995).

The level of analysis possible in model systems employed to study rhythmical behavior is extremely difficult to obtain in model systems used to study episodic movements. It is hard to study episodic behavior in slice because, unlike the cyclical activity underlying rhythmical behavior, an electrophysiological signature of motor command signals is missing. In fact, command signals may not occur when premotor neurons are separated from sensory inputs or, if they do occur, they may not be recognized because there is no movement to observe. Consequently, the goals of studies of episodic movements have been quite different, and the focus has been on molar, rather than molecular, issues. These include, for example, quantifying the relationship between neural and muscular activity as well as the direction, amplitude, and speed of movements, and understanding the neural basis of the variability in simple reaction time tasks. The effects of local perturbations of the spatial and temporal pattern of network activity on the latency, accuracy, speed, and trajectory of a movement inform us about how neurons with coarsely tuned movement fields can produce precise movements. Other studies focus on the transformations of sensory signals required to interface with motor command signals generated by neurons organized in motor maps, and understanding the manner in which the format of motor commands constrains the types of sensory processing that must occur. Considerable progress has been made in understanding the neural mechanisms underlying various types of motor plasticity. Studies of motor preparation examine neural activity that occurs well in advance of the executed movement. Increasingly, episodic behaviors are used to investigate cognitive factors (e.g., decision making, spatial attention, motor memory, motor intention, and movement cancellation) that influence motor control.

For those studying episodic movements, the route to detailed descriptions of the microcircuits involved and the biophysical and biochemical properties of the neurons in the circuits is usually indirect. For example, the network involved in

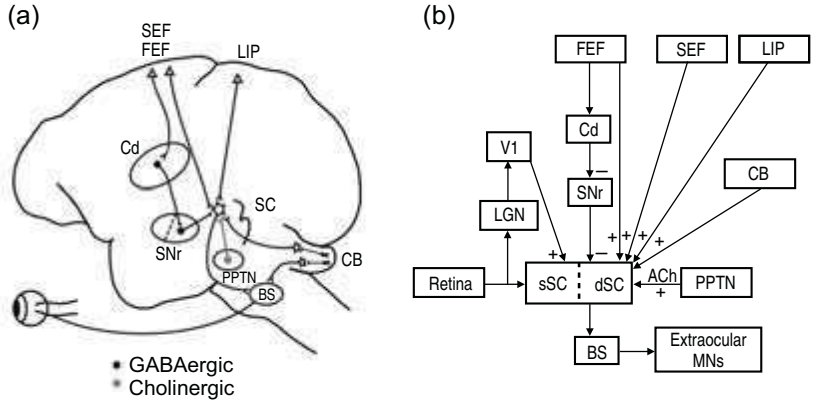

Figure 2.1 Widely distributed networks involved in the control of saccadic eye movements in the brain. BS: brainstem; CB: cerebellum; Cd: caudate nucleus; dSC: deep layer of the superior colliculus; FEF: frontal eye field; LGN: lateral geniculate nucleus; LIP: lateral intraparietal sulcus; MNs: motor neurons; PPTN: pedunculopontine tegmental nucleus; SC: superior colliculus; sSC: superficial layers of the superior colliculus; SEF: supplementary eye field; SNr: substantia nigra pars reticulata; V1: primary visual cortex; + at the arrowhead in (b) indicates glutamatergic excitatory connection; – indicates GABAergic inhibitory connection; ACh indicates cholinergic transmission.

controlling saccadic eye movements is distributed over many brain regions, including several lobes in the cerebral cortex, cerebellum, thalamus, basal ganglia, and widespread regions of brainstem (Figure 2.1). Progress is being made by removing brain regions containing critical components of the distributed network and studying, *in vitro*, the biophysical properties of morphological classes of neurons and the interactions between cells residing in different parts of local microcircuits. Hypotheses about the design principles of local microcircuits must be tested by putting the local circuit back into the *in vivo* system (not literally, of course) and testing the effects of microinjections of pharmacological agents or by using transgenic or knock-out animals. In contrast to rhythmical motor systems, in which the temporal pattern of a local microcircuit can be monitored while performing manipulations that have effects at cellular and molecular levels, cellular and molecular levels of explanation of episodic movements must be obtained by combining information from *in vivo* and *in vitro* experimental preparations. In this chapter, we present examples of this indirect approach. The *in vitro* data were acquired from experiments using slice preparations to study the intrinsic circuitry and biophysical properties of neurons in the superior colliculus (SC), a brain area critically involved in the initiation and execution of saccadic eye movements.

OVERVIEW OF THE ROLE OF THE SUPERIOR COLLICULUS IN THE CONTROL OF SACCADES

Here we provide a brief overview of the role of the SC in the initiation and control of saccadic eye movements (for recent reviews, see Isa 2002; Sparks 2002, 2004; Keller 2004). The SC, a laminated structure forming part of the roof of the midbrain, plays a critical role in triggering and organizing saccadic eye movements. The superficial layers are sensory in function. Cells have visual receptive fields and inputs arrive directly from the retina as well as from visual cortex. In contrast, the intermediate and deep layers have both sensory and motor functions. These layers receive visual, auditory, and somatosensory inputs from many cortical and subcortical regions (for references, see Sparks and Hartwich-Young 1989) and contain neurons generating commands for movements of the eyes, head, and (in some animals) the external ears. When recording in these deeper layers, various types of cells are encountered. Some display only sensory responses, others only motor-related activity; both sensory- and motor-related activity are present in the activity of a third general class of cells (see discussion below and Figure 2.6c–e). Many neurons in the deeper layers generate a high-frequency burst of spike activity that is tightly coupled to saccade onset; the burst begins 18–20 ms before saccade onset. Each neuron with a presaccadic burst has a movement field; that is, the cell discharges before a range of saccades that have particular directions and amplitudes. The burst of collicular cells is not related to moving the eye to a particular position in the orbit but to changes in eye position, which have a certain direction and a certain amplitude.

Saccade-related burst neurons are arranged topographically within the SC, and this organization forms the basis of the motor map revealed by microstimulation studies. In head-restrained animals, microstimulation in the deeper layers of the primate SC produces contralateral saccadic movements of both eyes with a latency of approximately 20–30 ms. Movements with upward directions are represented medially, and movements with downward directions are represented laterally. Stimulation of rostral regions produces small amplitude movements; larger movements occur with caudal stimulation. The site of stimulation within the colliculus determines the largest movement that can be produced; however, for any stimulation site, movement amplitude depends on the duration of the stimulation train. As train duration increases, movement amplitude increases monotonically until it reaches the site-specific limit. The peak velocity of an evoked movement is influenced by the frequency of stimulation: higher frequencies produce movements with higher velocities. In summary, these findings suggest that the site of collicular activity is the major determinant of saccade direction and amplitude but collicular activity must be sustained until the desired displacement is accomplished. The level of activity influences the speed of the movement.

At one level of analysis, the task of programming a particular saccade involves activating the appropriate region of the collicular map. Once output neurons in this region are driven into high-frequency burst mode, an accurate movement of the desired direction and amplitude will occur. The kinematic rules uncovered by motor psychophysics, such as the relationship between movement duration and amplitude, will be implemented downstream. The collicular command for accurate saccades is produced by neurons with broadly tuned receptive fields and coarsely tuned movement fields. The intrinsic and extrinsic anatomical connections are such that even crude, nonphysiological activation, such as electrical stimulation, is sculpted into a spatial and temporal pattern of activity that evokes movements indistinguishable from those produced by normal physiological inputs. How the pattern of intrinsic connections and the biophysical properties of collicular neurons may be involved in the initiation of saccades, and how accurate movements are produced by neurons with broadly tuned movement fields are discussed below.

CYTOARCHITECTURE OF LOCAL CIRCUITS IN THE SUPERIOR COLLICULUS

An early study by Langer and Lund (1974), using Golgi staining, revealed the cytoarchitecture of local circuits in the SC. According to this study, the local circuit in the superficial layers is composed of five neuron types: narrow-field vertical cells, wide-field vertical cells, piriform or stellate cells, horizontal cells, and marginal cells (Figure 2.2). Among these, wide-field vertical cells are major projection neurons connected to deeper layers of the SC (Mooney et al. 1988b) and lateral posterior nucleus of pulvinar (Lane et al. 1993). In addition, narrow-field vertical and marginal cells are also projection neurons targeted to the parabigeminal nucleus. Later immunohistochemical studies suggested that horizontal, stellate, and piriform cells are GABAergic based on the morphology of soma and proximal dendrites (Mize 1992). More recently, Endo et al. (2003a) showed that horizontal cells are GABAergic neurons by intracellular staining technique using biocytin in GAD67-GFP (green fluorescent protein) knock-in mice, in which GABAergic neurons are labeled with fluorescence of GFP.

The morphological characteristics of neurons in the intermediate layer are more heterogeneous. From the somatodendritic morphology, they are classified into multipolar-, pyramidal-, fusiform-, horizontal-, round-shaped, and wide-field vertical cells (Norita 1980; Ma et al. 1990). Their classification, however, appears to be less distinct than those in the superficial layer. The electrophysiological properties of SC neurons are also very heterogeneous. Based on the firing pattern to the depolarizing current step, they are classified into regular-spiking, late-spiking, burst-spiking, fast-spiking, and rapid adaptation types (Saito and Isa 1999). Among these, the regular-spiking type constitutes the majority of the neurons both in the superficial (50%; Endo and Isa, unpublished) and

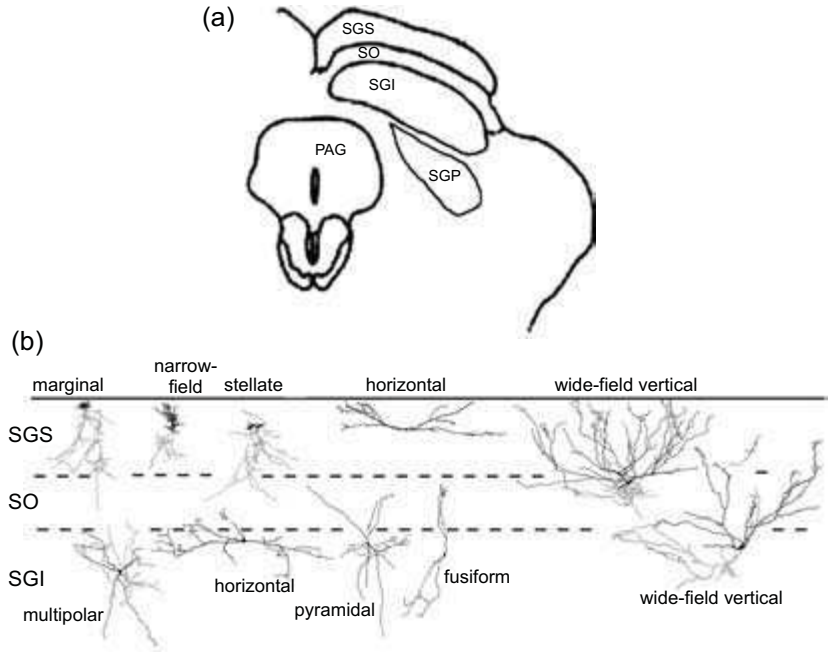

Figure 2.2 Morphological properties of neurons in the mammalian superior colliculus (SC). (a) Schematic drawing of lamina structure of SC. (b) Five major subclasses of neurons in the superficial layer (marginal cell, narrow-field vertical cells, stellate/piriform cells, horizontal cells, and wide-field vertical cells) and five major subclasses of neurons in the intermediate layer (multipolar cells, horizontal cells, pyramidal-shaped cells, fusiform cells, and wide-field vertical cells) are illustrated. Soma and dendrites are painted in black and axons in gray. SGS: stratum griseum superficiale; SO: stratum opticum; SGI: stratum griseum intermediale; SGP: stratum griseum profundum; PAG: periaqueductal gray. Modified from Isa and Saito (2001), with permission.

intermediate layer (50%; Saito and Isa 1999). In general, no clear correlation was observed between the electrophysiological properties and somatodendritic morphology of neurons, except expression of hyperpolarization-activated current (Ih) in wide-field vertical cells. Wide-field vertical cells project dendritic arbors into a horizontally wide area of the superficial layer. These neurons exclusively express Ih of large amplitude and fast time course (Lo et al. 1998; Saito and Isa 1999). A recent study by Endo et al. (2003b) showed that these cells express HCN1 channel molecules mainly in dendrites and that these Ih channels on the dendrites induce inward current continuously at the resting membrane potential and play a role in maintaining the membrane potential of distal dendrites always at the depolarized level. Further, Isa et al. (1998b) obtained evidence to suggest that dendritic action potentials are induced in the wide-field vertical cells. All together, the HCN channels on the dendrites of wide-field

vertical cells are supposed to contribute to lowering the threshold of generating dendritic action potentials in these neurons (Endo et al. 2003b).

The morphological and electrophysiological properties of deeper-layer neurons projecting to the contralateral paramedian pontine reticular formation (PPRF) were studied by whole-cell recordings from cells, which were retrogradely labeled by dextran conjugated Texas Red injected into the PPRF a few days before the experiments (Sooksawate et al. 2005). Among the 112 identified projection neurons in the SGI, regular-, burst-, late-, fast-spiking, and rapid inactivation type comprised of 73, 12, 11, 0, and 4%, respectively. Among the 76 projection neurons that were successfully stained with biocytin, multipolar, fusiform, pyramidal, horizontal, and round-shaped cells comprised 66, 13, 8, 11, and 3%, respectively. Thus, multipolar-shaped and regular-spiking neurons comprised the largest population of SGI neurons projecting to the contralateral PPRF.

INTERLAMINAR CONNECTION IN THE SUPERIOR COLLICULUS

In 1972, Robinson measured the vectors of saccades evoked by electrical stimulation of the SC and provided the first detailed description of the topographical map of saccade vectors in the deeper layers of the SC (Robinson 1972). In the same year, Schiller and Stryker (1972) demonstrated that the motor map was in register with the map of visual space represented in the superficial layers. Following these demonstrations, it was supposed that the superficial and deeper layers of the SC are organized in a columnar fashion by interlaminar connections that link them (Sprague 1975). However, the existence of such a connection and its functional implication has been a matter of long-lasting debate in the field. Evidence against the existence of interlaminar connections was presented by Edwards (1980) on the basis of anatomical studies. In addition, Mays and Sparks (1980) showed that during certain saccades, such as a saccade to the locus of a target that disappeared during the course of a preceding saccade ("double step saccade paradigm"), the bursting discharges of deeper-layer neurons need not be preceded by activation of overlying superficial layer neurons. They argued that the discharge of overlying visual cells is neither necessary nor sufficient to activate most intermediate layer neurons with saccade-related bursts. An exception to this general conclusion is the visually triggered movement cells (VTMs), first described by Mohler and Wurtz (1976). VTMs both have a visual receptive field and produce a saccade-related discharge when saccades are made to a visual target. These cells are silent during similar spontaneous saccades in the dark or light. VTMs do not require that a visual stimulus be present at the initiation of a saccade; it is only necessary that the target has recently appeared in an area of the visual field that corresponds to the movement field of the cell (Mays and Sparks 1980). VTMs have the properties expected if the visual

response of cells in intermediate layers were based on a superficial layer input. They discharge if, and only if, there has been a visual target in the region of the visual field to which a saccade is directed.

On the other hand, Maeda et al. (1979) showed that short latency excitatory postsynaptic potentials (EPSPs) can be induced in deeper-layer neurons following electrical stimulation of the optic nerve in anesthetized cats. In the late 1980s, the existence of an interlaminar connection was first demonstrated anatomically by Moschovakis and his colleagues in squirrel monkeys (Karabelas and Moschovakis 1985; Moschovakis et al. 1988) and by Rhoades and his colleagues in the hamster (Mooney et al. 1988a); it was later confirmed in additional species by Behan and Appell (1992), Lee and Hall (1995), and Hilbig and Schierwagen (1994). Further, in the late 1990s, the properties of the interlaminar connection was investigated in detail by using electrophysiological techniques in combination with intracellular staining techniques in slice preparations of the SC (Lee et al. 1997; Isa et al. 1998b). In this section, we summarize the observations obtained in slice studies and discuss the behavioral studies performed in nonhuman primates.

Figure 2.3d is a pictorial summary of the design of our experiments in slices obtained from rats 17–22 days of age. Stimulating electrodes were placed in the SGS and in the optic tract (OT) near the lateral border of the optic layer (SO) where the OT comprises a bundle of fibers. The responses of SGS, SO, and SGI neurons evoked by stimulation at these sites were recorded through patch pipettes. Single electrical pulses delivered through the OT electrode induced short latency monosynaptic EPSPs in both narrow-field vertical cells of the SGS, which project its axon extensively to the SO (Figure 2.3a) and in wide-field vertical cells of the SO, which projects to the SGI (Figure 2.3b). The EPSPs were moderately enhanced by application of $GABA_A$ receptor antagonist bicuculline (Figure 2.3a_4 and b_4) and were completely suppressed by application of the glutamate receptor antagonists CNQX and APV (Figure 2.3a_6 and b_6). These findings demonstrate that both SGS and SO neurons receive glutamatergic monosynaptic excitation from the OT. To judge from their longer latency and fluctuating onset latencies, the EPSPs evoked in SGI neurons following stimulation of the OT (Figure 2.3c_2 and c_3) were di- or oligosynaptic. These responses were markedly enhanced by application of $GABA_A$ receptor antagonists, such as bicuculline (Figure 2.3c_4) or SR95531; the bursting spike discharges evoked in the SGI cells were superimposed on large clusters of EPSPs, which could last longer than 1 s, even when single brief electrical pulses were delivered through the OT electrode. The long-lasting depolarization and bursting spike discharges were evoked in an all-or-nothing fashion at threshold stimulus intensities (Figure 2.3c_5). Stimulation of the SGS induced monosynaptic EPSPs in SGI neurons, which were again amplified into bursting spike discharges superimposed on the long-lasting depolarization following application of bicuculline (Figure 2.3$e_{1–4}$). These results confirm the existence of an excitatory pathway from the

OT to SGI neurons, presumably mediated by SGS or SO neurons, as previously demonstrated by Lee et al. (1997) using whole-cell recording in SC slices from the tree shrew. They also argued that release from $GABA_A$ receptor-mediated inhibition is needed for SGI neurons to exhibit vigorous bursting responses to uncaging of glutamate by UV light (Pettit et al. 1999).

These observations obtained in slice preparations were confirmed in *in vivo* experiments. The bursting responses evoked in SGI neurons by OT stimulation were observed following the blockade of $GABA_A$ receptor-mediated synaptic transmission in anesthetized rats (Katsuta and Isa 2003) and more recently in anesthetized monkeys (Nikitin, Kato, and Isa, unpublished).

Thus, the existence of the interlaminar connection has been demonstrated, and the signal transmission through the pathway is enhanced by disinhibition from $GABA_A$ receptor-mediated inhibition. The obvious question is thus: What use does the interlaminar connection fulfill? One hypothesis states that the interlaminar connection is utilized for execution of express saccades with extremely short reaction times (ranging from 80–120 ms). In earlier studies by Hikosaka and Wurtz (1985a), the effect of injection of bicuculline into the SC on visually guided saccades in monkeys was investigated. Their observation of significant reductions in saccadic reaction times, and especially increase in frequency of express saccades, was confirmed by Sparks et al. (1990). In more recent studies, Isa and colleagues tested the effect of other neurotransmitters that modulate the signal transmission through the interlaminar connection.

In *in vitro* experiments, neurotransmitters were explored that might modulate the threshold above which SGI neurons emit bursts in response to synaptic drive. Acetylcholine (ACh) is a good candidate for modulating the threshold of SGI cells, as it is employed by pathways to the SGI from the parabrachial region of the midbrain (the pedunculopontine and laterodorsal tegmental nuclei). In rat slice experiments, Isa et al. (1998a) found that application of ACh primarily activates $\alpha 4\beta 2$ type nicotinic ACh receptors (nAChRs) and M3 type muscarinic receptors of SGI neurons and causes their depolarization (Sooksawate and Isa, in preparation). Isa et al. further observed that depolarization caused by nAChR activation lowered the threshold for eliciting bursting response to excitatory synaptic inputs, such as those from the OT (Figure 2.4) and SGS (not illustrated). Based on the observations of the effect of nAChR activation on SGI neurons, Isa and colleagues microinjected nicotine locally into the SGI of monkeys while they were engaged in a visually guided saccade task (Aizawa et al. 1999; Watanabe et al. 2005). As shown in Figure 2.5, nicotine induced a stepwise reduction in saccade reaction time (SRT), from that of regular saccades (150–160 ms) to that of express saccades (about 100 ms). Interestingly, the SRTs exhibited a clear bimodal distribution after the onset of nicotine injection and even during recovery from it. As shown in Figure 2.5, few saccades of intermediate SRTs were elicited. Increase in the dose of nicotine caused no further reduction in SRTs. These results suggest that express saccades may be produced by the local

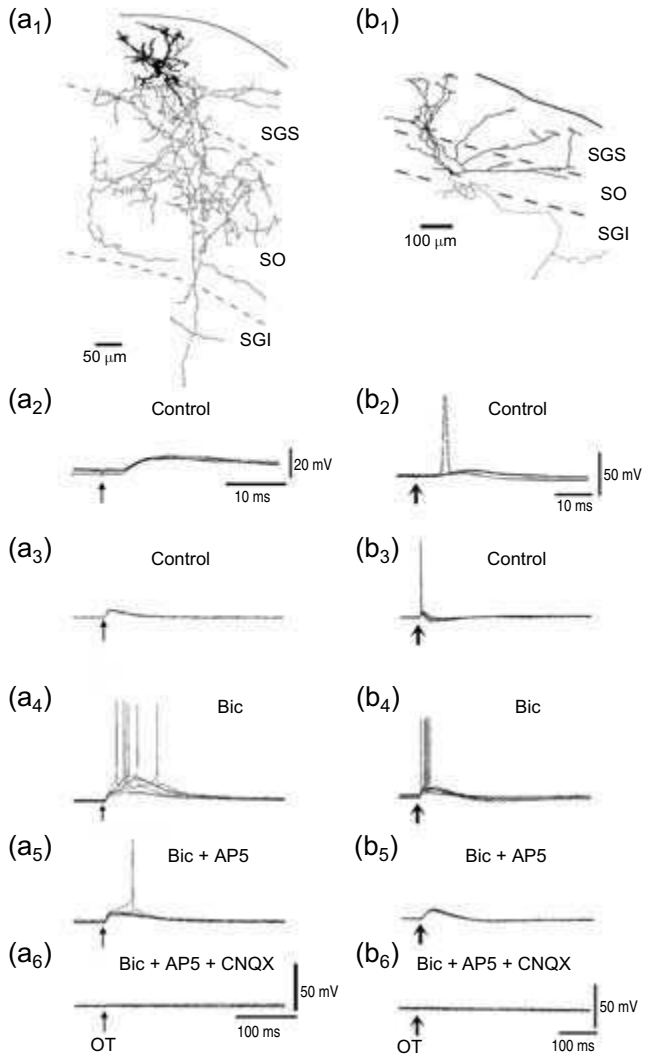

Figure 2.3 Effect of the optic tract (OT) and SGS stimulation on SGS, SO, and SGI neurons studied in the slice preparation of the rat SC. (d) Experimental arrangement: Bipolar stimulating electrode was placed either in the most dorsal portion of the SO to stimulate the bundle of optic fibers or in the SGS. In the former, a cut was added to the slice to eliminate the possibility of current spread outside the OT to activate the SGI neurons. (a) Recording from a narrow-field vertical cell in the SGS: (a_1) Morphology of the neuron stained with intracellular injection of biocytin. Soma and dendrites are painted in black; axonal branches in gray. (a_2) and (a_3) The effect of the OT stimulation in the control solution with different time sweep. (a_4) During application of 10 μM bicuculline (Bic). (a_5) During application if Bic plus 50 μM AP5. (a_6) Additional application of 10 μM CNQX. (b) Recording from a wide-field vertical cell in the SO. The same arrangement as (a).

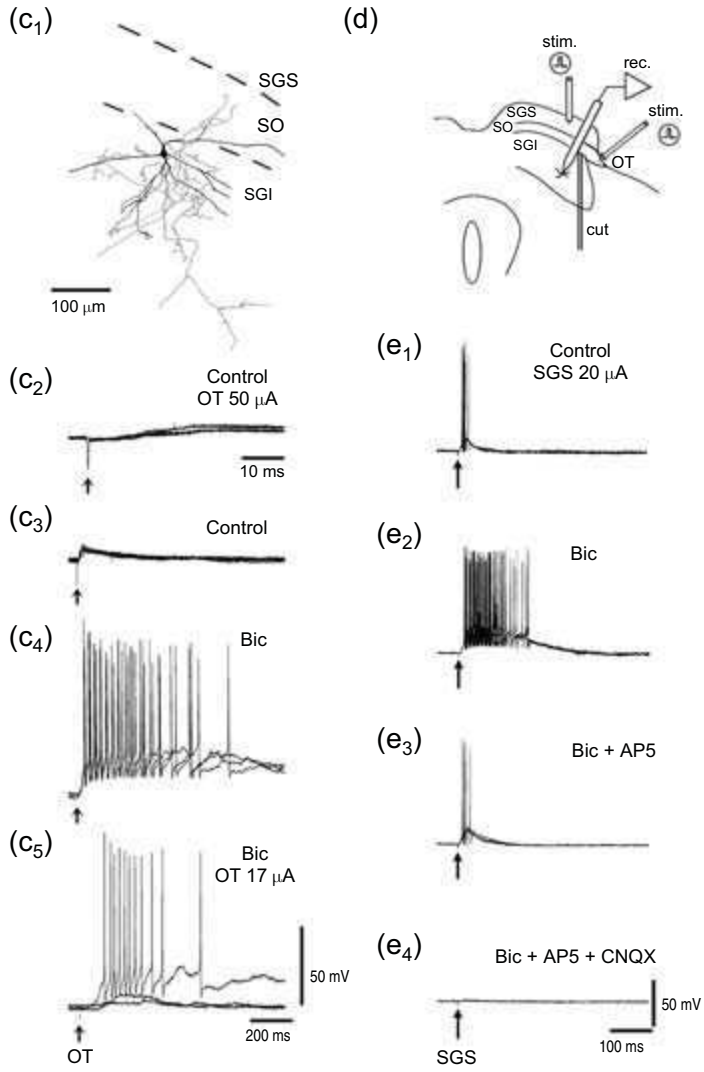

Figure 2.3 (*continued*) (c_1) Morphology of the neuron stained with intracellular injection of biocytin. (c_2) and (c_3) The effect of the OT stimulation in the control solution with different time sweep. (c_4) During application of 10 μM Bic. The stimulus strength was suprathreshold (50 μA). (c_5) At the threshold current intensity (17 μA), the bursting response occurred in an all-or-none manner. (e) Effects of stimulation of the SGS onto a SGI neuron in the control solution (e_1), after application of bicuculline (10 μM) (e_2) and application of 50 μM APV, which largely abolished the long-lasting depolarization (e_3) and additional application of 10 μM CNQX completely abolished the EPSPs (e_4). From Isa et al. (1998) and Isa (2002), with permission.

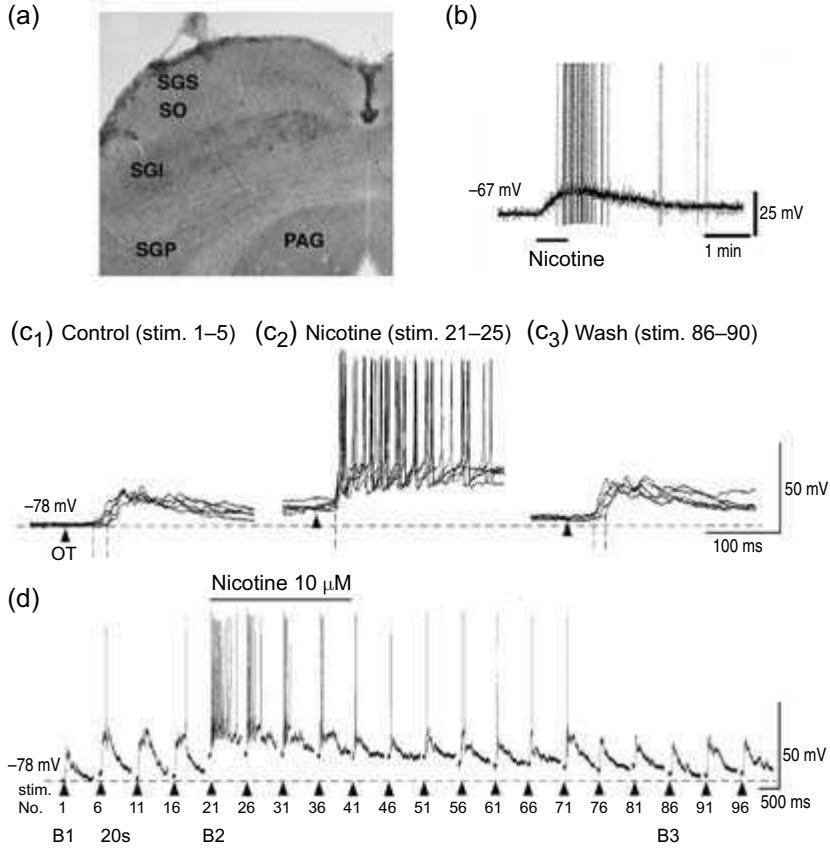

Figure 2.4 Cholinergic effect on SGI neurons. (a) Immunohistochemical staining against choline acetyltransferase. Afferent fibers terminating in the SGI are densely stained. (b) Depolarization induced by application of 30 μM nicotine. (c) Effect of nicotine application (30 μM) to EPSPs induced by stimulation of SGS: (c_1) control responses; (c_2) during nicotine application; (c_3) after nicotine washout. (d) Time course of effect of nicotine application.

increases in the excitability of SGI neurons. They also suggest that the nicotine-induced movements rely on the existence of a short transmission time pathway, probably within the SC, for their execution. The interlaminar projection of the superficial to the deeper tectal layers provides such a pathway when the signal flow through it is enabled, so that visual signals that reach the SGS can be transformed into the bursts of action potentials that comprise motor commands. This hypothesis is supported by observation of neuronal activities in the superficial and deeper-layer neurons of the SC during express regular saccades (Figure 2.6).

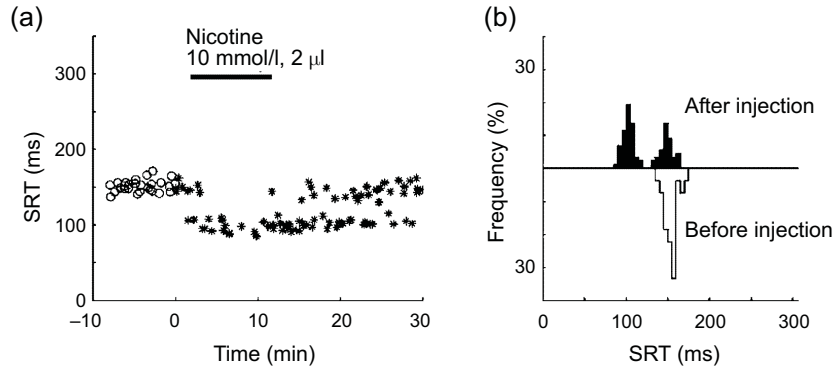

Figure 2.5 Effects of nicotine injection (10 mmol/l, 2 μl) into the monkey SC on saccade reaction time (SRT) during gap (170 ms) visually guided saccade paradigms. Amplitude of saccades was 7° in horizontal and 7° in vertical directions. A horizontal bar in (a) indicates period of nicotine injection (10 min). (a) SRTs are plotted against time. Open circles represent trials before injection; asterisks indicate trials during and after nicotine injection. (b) Distribution of saccadic reaction times before (lower) and after injection of nicotine. From Aizawa et al. (1999), with permission.

Figure 2.6a illustrates the discharge of a superficial layer (SGS) neuron and an intermediate layer (SGI) neuron for "regular saccades" (SRTs > 150 ms) in the no-gap condition. Figure 2.6b illustrates the discharge of the same neurons for express saccades (in a gap task with period equal to 170 ms). As shown here, the discharge of the SGS neuron was virtually the same for regular and express saccades. In both cases, it exhibited phasic visual responses, with latencies ranging between 40 and 50 ms. Thus, the execution of express saccades cannot be ascribed to the modification of the discharge of SGS neurons. On the other hand, the SGI neuron did not exhibit the same pattern of activation for regular and express saccades. For regular saccades, the SGI neuron exhibited a weak discharge with a latency of 40–50 ms, that is, similar to that of the visual responses of SGS neurons. This weak, phasic, "visual response" did not appear to be strong enough, by itself, to trigger the saccade burst generator circuits downstream of the SC. Then, 50–80 ms later, the SGI neuron exhibited a strong burst of spikes ("premotor burst"), which appeared to trigger saccades. In contrast, for express saccades, a gradual increase of discharge was observed during the gap period (arrow in Figure 2.6b). The buildup was followed by a strong unimodal burst of spikes with latency equal to that of "visual responses" that occur prior to regular saccades.

The activity of cells in the intermediate layers displaying visual, visuomotor, and motor bursts are shown during regular saccades and during trials with gap intervals of 150 and 300 ms (Figure 2.6c–e). The trials are ranked according to reaction time. The latency of the visual response of visual and visuomotor cells

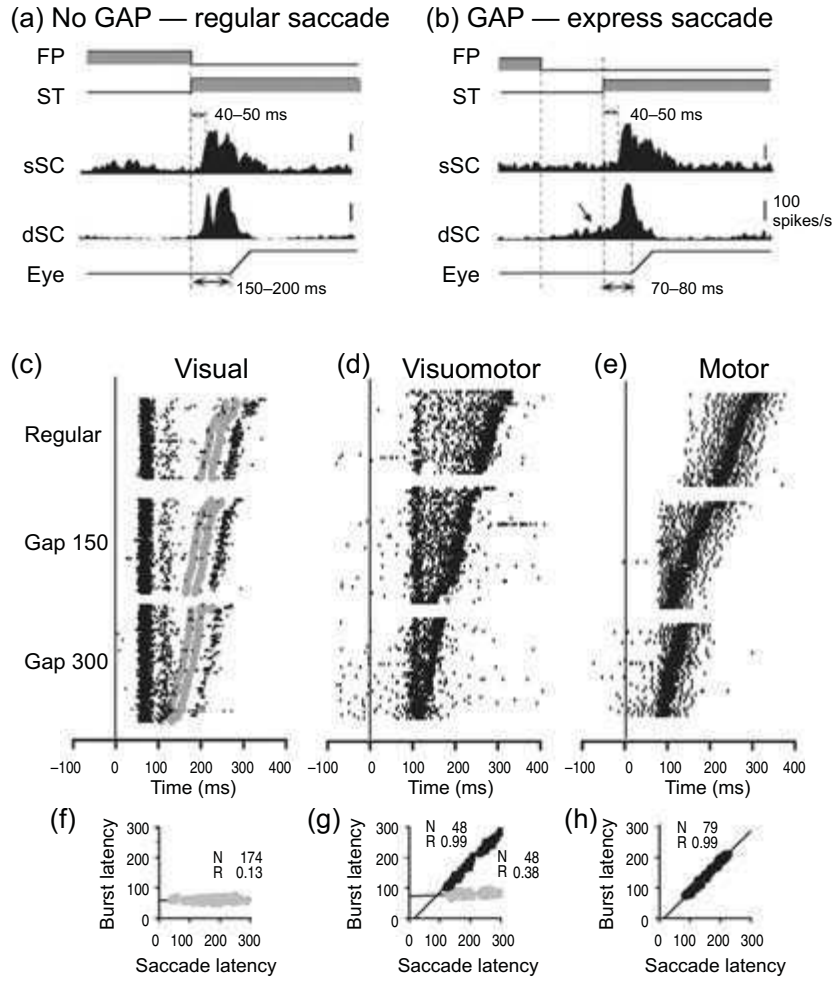

Figure 2.6 Activity of an sSC neuron and a dSC neuron during regular saccades in the no-gap paradigm (a), and express saccades in the gap paradigm (gap period lasting for 170 ms) (b) in the monkey. Spike density functions of exemplary neurons are shown. FP: presentation of fixation point; ST: presentation of saccade target; Eye: eye position. (a)–(b) from Isa (2003) with permission. (c)–(e): Raster display of the activity of a visual (c), visuomotor (d), and motor (e) cell during regular saccade trials and 150 and 300 ms gap trials. Each tick mark represents the occurrence of an action potential and each row of tick marks represents the activity observed during a single trial. The broad, gray tick marks in panel (c) represent saccade onset and offset. The visual cell displayed a second period of activation associated with the target image motion on the retina during the saccade. During gap trials, the fixation stimulus was extinguished 150 or 300 ms before the target appeared. The trials are aligned on target onset (the vertical line), and each set of trials is arranged in descending order of saccadic reaction time. *Caption continues on next page.*

is unrelated to reaction time (Figure 2.6f, g) whereas the motor burst of visuomotor and motor cells is highly correlated with reaction time over the full range of saccadic latencies. For saccades in the express range, the visual and motor bursts of visuomotor cells merge and the onset or offset of either the visual or the motor burst cannot be defined. Collectively, these data suggest that the signal transmission through the interlaminar connection modifies the excitability of presaccadic burst neurons in the SGI and induces an early burst in the case of express saccades. However, the direct evidence to show that the signal is actually transmitted from the superficial layer to deeper layer is still lacking, and the neuronal mechanisms of express saccades are still unclear.

NEURONAL MECHANISM FOR GENERATION OF PRESACCADIC BURST

When and where to make saccades has been intensively studied as a model system for studying the neural implementation of decision-making processes by the brain. Most models assume that where (the direction and amplitude of the movement) to make saccades is determined by the location of the neuronal population engaged in the saccade execution on the topographical map in the SC. On the other hand, the reaction time interval between the onset of visual stimulation and saccade onset is quite variable, ranging between 100 and 500 ms. To explain this variation, many models have been proposed and, among them, the accumulator model has been evaluated in terms of neural implementation.

In the accumulator model, the initiation of saccadic eye movement is determined by the timing of when the gradual rise of the decision signal exceeds a certain threshold level for saccade generation. Neural activities of several saccade-related structures in the brain were investigated based on this model in nonhuman primates. Among these, those in the deeper layer of the SC well represent a typical pattern that fits the model. Under the condition where the subject can anticipate the forthcoming target location, the gradual increase in activity can be observed in the deeper-layer neurons ("prelude activity"), and the sudden change of activity to a burst is supposed to trigger the initiation of saccades. The

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Figure 2.6 (*continued*) Delayed saccade trials, step trials, 150 ms gap trials, and 300 ms gap trials were randomly interleaved during data collection. (f) Visual burst latency as a function of saccade latency for the visual cell illustrated in (c). (g) Visual (gray) and motor (black) burst latency as a function of saccade latency for the visuomotor cell illustrated in (d). Data points for express saccades with the shortest latencies are not illustrated because the onset of the visual and motor bursts could not be defined. (h) Motor burst latency as a function of saccade latency for the cell illustrated in (e). Saccade onset was defined using a velocity criterion (40°/s). Burst onset was defined as the first of four consecutive instantaneous frequency values greater than 300 spikes/s. (c)–(h) from Sparks et al. (2000), with permission.

high-frequency burst of collicular neurons is tightly coupled to saccade onset (leading the movement by 18–20 ms; see Figure 2.6h). Moreover, the occurrence, or lack thereof, of the high-frequency burst can be used to predict perfectly the behavior of a monkey performing a behavioral task in which the probability of saccade initiation is manipulated by varying target duration (Sparks 1978). The hypothesis (Sparks 1978; see also Keller 1979) that, in normal animals, the motor burst of collicular neurons serves as a signal, which triggers saccade onset, must now be extended to express saccades (Sparks et al. 2000). The prelude activity of the deeper-layer neurons predicts the vector of forthcoming saccade (Glimcher and Sparks 1992), and the higher the activity, the shorter the saccadic reaction times (Dorris et al. 1997; Sparks et al. 2000). This suggests that the initiation of the presaccadic burst is determined by the timing when the prelude activity exceeds "the threshold." The question therefore arises as to whether the threshold effect is implemented by mechanisms intrinsic to the local circuit of the SC or whether it merely reflects events occurring outside the SC. *In vitro* slice preparation of the SC that is separated from other brain structures can be a powerful tool for addressing this issue, since this preparation allows a description of the properties of the intrinsic circuit of the SC. The bursting responses of neurons may arise from several alternative mechanisms, either at intrinsic membrane properties of individual neurons or by the structure of the circuit. As the first step to explore this issue, it was essential to know the intrinsic membrane properties of individual identified tectofugal cells. It was found that most of the crossed tectoreticular SGI cells identified by retrograde labeling with a fluorescent tracer exhibited regular spiking properties and a quasi-linear f–I relationship (Sooksawate et al. 2005). Also, only a small population of tectofugal neurons exhibited burst spiking or plateau potentials. On the other hand, as shown in Figure 2.4d, the bursts emitted by SGI neurons, in response to stimulation of the SGS in the presence of bicuculline, were suppressed by application of 50 μM APV, and thus the bursts depended on activation of NMDA-type glutamate receptors. It is well known that NMDA receptors have a J-shaped current–voltage relationship. Due to Mg^{2+}-block, the NMDA-type glutamate receptors admit inward currents only when the cell is sufficiently depolarized. Once the membrane potential exceeds the value necessary for activation of the NMDA receptor, a regenerative process ensues, which further enhances their depolarization. Such nonlinear activation of NMDA receptors can account for the all-or-nothing character of the bursts emitted by SGI neurons.

Presaccadic neurons of the primate SC are known to have recurrent collaterals, which ramify in the neighborhood of the parent somata (Moschovakis et al. 1988). To further investigate whether a local circuit including such neurons could support their long-lasting depolarization and bursting activity, Saito and Isa (2003) obtained simultaneous, dual, whole-cell records from pairs of adjacent SGI neurons. Figure 2.7 illustrates an example obtained from a pair of neurons horizontally separated by less than 100 μm. The existence of direct

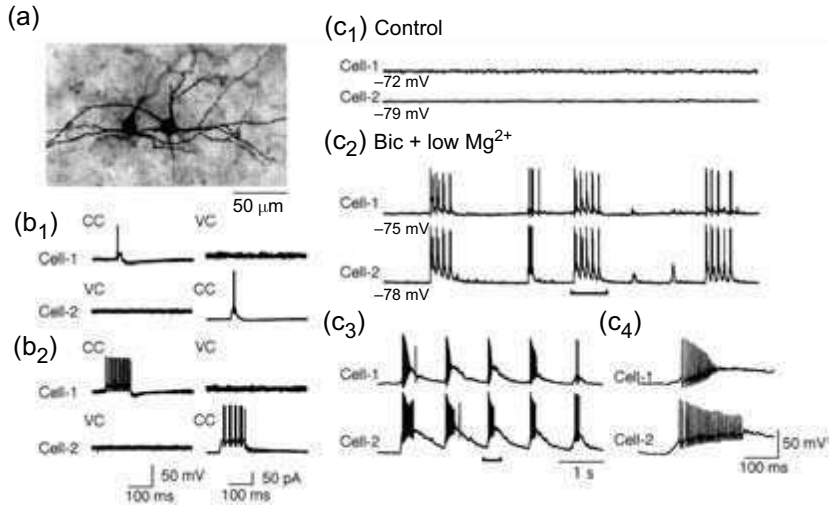

Figure 2.7 Simultaneous recordings from a pair of SGI neurons. (a) Photomicrograph of the pair of recorded SGI neurons (injected with biocytin intracellularly). (b) Recordings of current responses from one cell in voltage-clamp mode (VC) after induction of a single (b_1) or several (b_2) action potential(s) in the other cell by current injection in current-clamp mode (CC). (c) Spontaneous membrane potentials in control solution (c_1), in the presence of 10 μM Bic and low (0.1 mM) Mg^{2+} (c_2). (c_3) shows faster-sweep records of segments underlined in (c_2); (c_4) shows faster-sweep records of segment underlined in (c_3). From Saito and Isa (2003), with permission.

connections between the two cells was evaluated by measuring the responses of one of the cells to current injected into the other in order to induce its single or repetitive firing. In this manner, we established that the two neurons (Figure 2.7a, b) were not directly connected with each other. This result applied to most of the cases we examined (over 95%). When we applied 10 μM bicuculline (or SR95531) to block $GABA_A$ receptors and reduced the extracellular concentration of Mg^{2+} from 1.0–0.1 mM to enhance the NMDA receptor-mediated responses, the SGI neurons exhibited bursting spike activity superimposed on repetitive, spontaneous, depolarizing potentials. Interestingly, the spontaneous depolarization and the bursting spike activity occurred almost simultaneously in both neurons. Since the spiking discharges of two adjacent presaccadic burst neurons are synchronous, synchronization of SC neuron discharges could underlie the generation of their presaccadic bursts *in vivo*. It was further found that activation of NMDA-type glutamate receptors is essential for such synchronous depolarization to occur since it was completely abolished by APV. A small piece of the intermediate layer (0.3 mm thick and measuring about 0.5 mm by 1 mm in the dorsoventral and mediolateral directions, respectively) was adequate to support the synchronous depolarization and bursting of pairs of neurons.

NEURONAL MECHANISM OF POPULATION CODING

The movement field of an individual burst neuron in the deeper layer is broad and coarsely tuned. The activity of a single neuron is ambiguous with respect to coding the vector of the executed saccade because similar bursts are generated for movements having quite different directions and amplitudes (Sparks and Mays 1980). Since each cell discharges before a wide range of movements, a large population of cells is active before each movement. Precise control of saccade direction and amplitude is assumed to be attained by averaging the activities of a large population of deeper-layer neurons distributed across a wide area of the SC map (Lee et al. 1988). An example of data consistent with the vector averaging hypothesis is presented in Figure 2.8. Simultaneous visually induced activity of neurons in one part of the motor map and electrical stimulation-evoked activity of neurons in another part of the motor map produce saccades with amplitudes and directions that can be described as a vector average output. Current models of the saccadic system (Keller 2004) that can account for various behavioral phenomena (e.g., vector averaging) use intracollicular connections and recurrent feedback from output cells to shape the spatiotemporal pattern of the population of collicular cells active before and during a saccade. For example, recurrent excitatory feedback from tectoreticular burst neurons is used to help generate a large region of active neurons surrounding the locus of the visual input to the motor layer. Other models implement strong winner-take-all behavior in a distributed neural map by the strength and extent of the lateral inhibitory connections (see Keller 2004). Only recently have methods become available for actually assessing the extent to which widespread excitatory and inhibitory connections exist between different regions of the collicular map. Results of experiments assessing intrinsic excitatory connections in the collicular network are described below. Moschovakis et al. (1998) studied the morphology of burst neurons in the deeper layer in squirrel monkeys by intracellular staining with horseradish peroxidase and showed that tectal long-lead burst neurons in the deeper layer issue a large number of axon collaterals in the deeper layer and suggested the existence of tight local excitatory connections in the deeper layer. A more recent study by Hall and colleagues showed local stimulation of SGI neurons, by uncaging of caged glutamate compound, induced excitation of neurons in horizontally distant neurons (Helms et al. 2004). Further, a recent study by Saito and Isa (2004) studied the lateral excitatory connection in the SGI by making dual whole-cell recordings from pairs of SGI neurons. As shown above, when whole-cell recordings were performed from two adjacent SGI neurons and we added bicuculline and reduced extracellular Mg^{2+} concentration to 0.1 mM, the two cells exhibit spontaneous depolarization and bursts of firings synchronously. Such spontaneous synchronization could be observed when QX-314 was applied to the intracellular solution to block spike generation. Because the amplitudes of

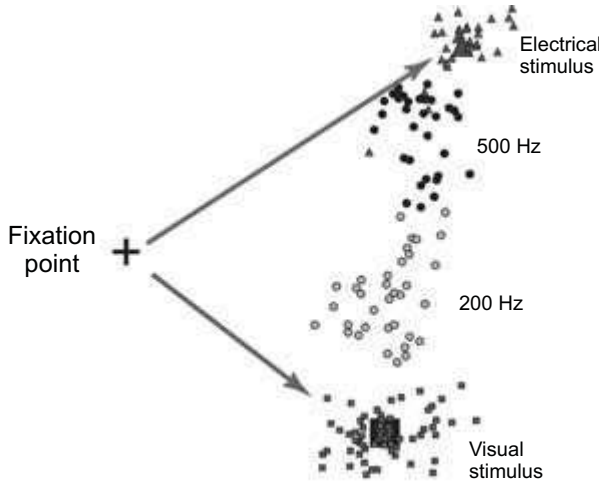

Figure 2.8 Effects of simultaneous activation of two regions of the SC. After the monkey maintained fixation of a centrally located visual fixation stimulus (+) for a variable period of time (200–800 ms), an eccentric target (large box) was flashed for 50 ms 9° to the right and 7° downward. The endpoints of saccades to this dim, briefly flashed target (squares) were variable in amplitude and direction. The endpoints of saccades evoked on trials in which microstimulation trains were delivered in the absence of an eccentric visual target are shown as triangles. The stimulation-evoked movements were also variable for these near-threshold stimulation trains (6 microamps current, pulse duration 0.5 ms). Randomly, on 30–50% of the trials, a train of microstimulation pulses was delivered after a variable delay (2–175 ms) following the offset of the visual target. The stimulation train continued until an eye movement occurred. Endpoint of saccades occurring on trials with both visual and electrical stimulation with 200 Hz trains are represented as gray circles. Robust averaging effects were observed. With combined visual and electrical stimulation, saccades were directed to points intermediate between the visual and stimulation-evoked movements. On trials in which the frequency of the stimulation train was 500 Hz (black circles), the endpoints of saccades were further displaced from the endpoints of visual saccades and closer to the endpoints of electrical saccades. Saccades with endpoints at almost any location on the line connecting the cluster of points observed on visual trials and the cluster on stimulation trials can be obtained by varying the number of pulses and the frequency of stimulation.

depolarization were not always identical between a pair of neurons, the phase plots were made from plots of amplitudes normalized to the maximum amplitude of depolarization of each neuron (Figure 2.9b_1). The "percent of synchronous depolarization (PSD)" was defined as the sum of the number of plots with the values of normalized amplitude of both neurons greater than 0.5 (plots in area II of Figure 2.9b_2) divided by the sum of the number of plots in areas I, II, and III (Figure 2.9 b_2). In addition, the correlation coefficient of the plots was measured. The PSD and coefficient of the correlation shown in Figure 2.9 b_1 was calculated to be 69.8 and 0.91, respectively, which indicated a high degree

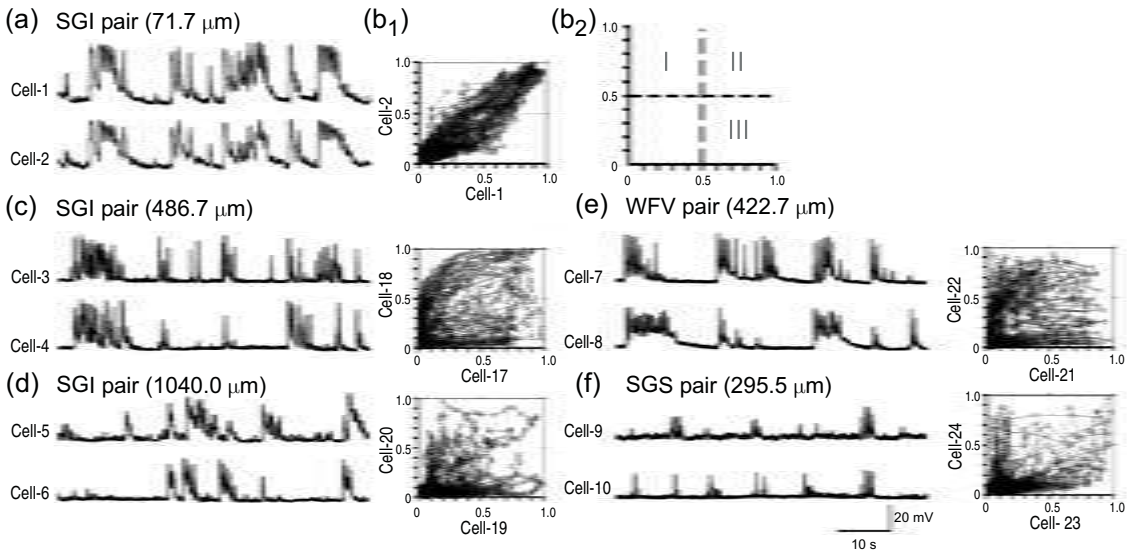

Figure 2.9 Comparison of the extent of lateral excitatory interaction among different layers of the SC. Relationship between the parameters (percent of synchronous depolarization and correlation coefficient) and the distance between the recorded neurons in SC are plotted for neuron pairs of SGI neurons, wide-field vertical (WFV) cells in the SO, and SGI neurons. (a) (c), and (d): Spontaneous membrane potentials of SGI neurons in the presence of 10 μM Bic and low Mg^{2+} (0.1 mM). The distances between the recorded neurons in (a) (cell 1 and 2), (c) (cell 3 and 4), and (d) (cell 5 and 6) were 71.7, 486.7, and 1040.0 μm, respectively. Phase plots of normalized membrane potentials of cell 1 against those of cell 2 (obtained from recording for 4 min) is shown in (b_1). In (b_2), phase plot was divided into four quadrants and the number of points in quadrant II was divided by those in I plus II plus III to evaluate PSD. (e) and (f): Spontaneous membrane potentials of a pair of WFV cells (cell 7 and 8, distance = 422.7 μm) and a pair of SGS neurons (cell-9 and cell-10, distance = 295.5 μm) in the presence of Bic and low Mg^{2+}. In (c)–(f), the phase plots are shown on the right. (f): Spontaneous membrane potentials of a pair of WFV cells (cell-7 and cell-8, distance = 422.7 μm) and a pair of SGS neurons (cell-9 and cell-10, distance = 295.5 μm) in the presence of Bic and low Mg^{2+}. In (c)–(f), the phase plots are shown on the right.

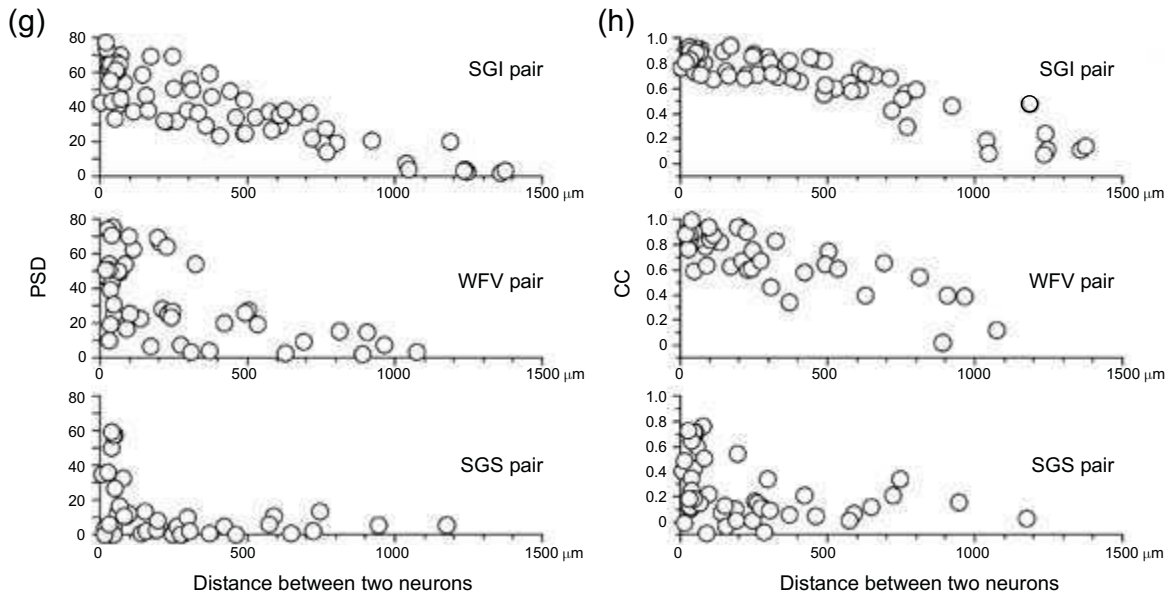

Figure 2.9 (*continued*) (g) and (h): Plots of the percentage of synchronous depolarization (PSD) and the correlation coefficient (CC) are shown against the distance between the recorded neurons in SGI (upper panel, n = 64), wide-field vertical (WFV) (middle panel, n = 44), and SGS (bottom panel, n = 45) pairs. Modified from Saito and Isa (2004), with permission.

of correlation. Then, to investigate the intensity of lateral excitatory interaction, we measured the PSD and correlation coefficient of cell pairs with various horizontal distances in the SGI. As shown (Figure 2.9g, h) the degree of synchronization was reduced as the distance increased and reached virtually zero when the distance of the recorded pairs was 1 mm. We made a similar analysis on pairs of wide-field vertical cells in the SO and pairs of SGS neurons (non-wide-field vertical cells). Wide-field vertical cells in the SO exhibited virtually similar degree of synchronization in relation to the distance. In contrast to these subclasses of neurons, pairs of SGS neurons rarely showed synchronization and even, if they exhibited synchronicity, it is observed only when the distance of the pairs was less than 100 μm. These results indicate that the lateral excitatory connection is intense and widespread in the SGI and among wide-field vertical cells in the SO, whereas SGS neurons have lateral excitatory connection only among a cell population in the close proximity. In other words, the SGS neurons compose horizontally narrow columnar-like structures, which may correspond to visual receptive fields, whereas wide-field vertical cells in the SO and SGI neurons do not compose distinct columnar structure.

SUMMARY

As illustrated, slice preparation has become an important complement to behavioral and electrophysiological studies in alert animals. A few of the advantages of the method and some of the new findings that have emerged from slice preparations were highlighted here. Descriptions of the cytoarchitecture of brain areas involved in ocular motility and of the intrinsic circuitry of these areas are more readily acquired using slice preparations and are more definitive than descriptions produced using more conventional techniques. Indeed, studies in slice resolved the long-standing uncertainty about direct communication between superficial and deep layers of SC. This uncertainty existed because of the possibility of labeling or stimulating fibers of passage rather than neurons. Intracellular recordings can be used to classify neurons and electrophysiological cell types can be correlated with morphological categories. For example, Ih is an electrophysiological signature of wide-field vertical cells in SC. The synchronicity of the activity in different neurons, the mechanisms responsible for the synchronicity, and the spatial extent of synchronous activity can be more easily determined *in vitro* than in *in vivo*. The transmitters and receptors used for communication between layers or subregions can be identified. An excitatory pathway from the OT to SGI neurons is now well established, and signal transmission over this route is enhanced by disinhibition from $GABA_A$ receptor-mediated inhibition. The threshold for the bursting response of SGI neurons to excitatory synaptic inputs can be lowered by depolarization produced by nicotinic ACh receptors. Such findings have implications for observations made in alert animals performing eye movement tasks. For example, extracellular records of

the activity of saccade-related burst neurons in monkey often show a distinct pattern. The prelude of activity that precedes the high-frequency burst usually maintains a stable frequency of 100 Hz or less for a brief period, and there is a sudden transition to a high-frequency (800–1000 Hz), short-duration burst. Based on data from slice, the transition to the all-or-nothing high-frequency burst may depend on activation of NMDA-type glutamate receptors. However, the behavioral effects of blocking NMDA-type receptors in the output layers are unknown.

LIMITATIONS OF CURRENT METHODS FOR STUDYING EPISODIC MOVEMENTS

In many of the model systems used to study rhythmical movements, the behavior to be explained (locomotion, respiration, mastication) is generated by the same organism being studied in the slice or dish. Studies of the stomatogastric ganglion seek explanations for behavior observed in crustaceans. For the oculomotor system, cellular and molecular data usually come from slices of the mouse or rat brain, whereas data concerning the movements to be explained usually were collected from nonhuman primates. This discrepancy will be eliminated as we acquire more information about the properties of eye movements generated by rodents (van Alphen et al. 2001; Sakatani and Isa 2004). The link between behavior and the properties of cells and circuits will be more direct if both types of data are obtained from the same animal. However, in the case of eye movements, the strategy of selecting the experimental preparation that permits the use of the most powerful analytical tools available yields different choices: The mouse will be selected for studies of cellular and molecular mechanisms, the monkey for behavioral studies (rodents are nocturnal and do not have a fovea; thus, the level of cognitive control over eye movements may be very different in rodents and monkeys). Cellular and molecular mechanisms of neuronal and circuit behaviors will develop quickly in mouse studies, but experiments designed to test the generality of these findings and putative mechanisms in animals with other visual demands on the oculomotor system will be highly valued.

An additional limitation of current isolated preparations is the inability to study the interactions of visual, auditory, and somatosenory stimuli. Because sensory input is not preserved, cellular and molecular mechanisms involved in converting sensory signals organized in different coordinate frames into the same reference frame, so that they can share a common motor circuitry, cannot be examined. Mechanisms involved in the dynamic mapping of auditory receptive fields (Jay and Sparks 1987) are also unlikely to emerge from data obtained in slice.

THE NEED FOR ADDITIONAL ISOLATED PREPARATIONS

The location of the active cells in the collicular map is the major determinant of the direction and amplitude of a saccade. The high-frequency burst generated by collicular output neurons has all the characteristics of the signal needed to trigger a saccade. However, the format of the motor command signals observed downstream are very different. Important transformations of collicular signals must occur. In contrast to the predominantly place-coded signal found in SC, the command for a movement in downstream structures is coded temporally. Excitatory burst neurons (EBNs) in the pons generate activity that controls the horizontal component of a saccade. In the midbrain, EBNs produce activity responsible for vertical and torsional components of saccades. The temporal features of a saccade (duration and speed) are determined by temporal characteristics of the activity of EBNs. Saccade duration is tightly coupled to burst duration, and burst rate is highly correlated with saccade velocity. Motor neurons also generate a temporally modulated command signal. The initial burst of activity (the pulse) is followed by a sustained tonic level of activity (the step). Amplitude and speed of a saccade are determined by the size and duration of the pulse.

How are the predominantly place-coded command signals observed in SC translated into the temporally coded signals carried by downstream premotor and motor neurons? This question is called the spatial-to-temporal transform problem (STTP). With respect to the neural control of saccades, STTP is one of the most important problems remaining to be solved.

Some of the processing required for the STTP may occur within the SC. The burst of activity of neurons in caudal regions of SC, which precedes large gaze shifts accomplished by a combination of eye and head movements, has a much lower peak frequency than the high-frequency bursts of rostral cells associated with movements of smaller amplitudes (Freedman and Sparks 1997). A change in the properties of the saccade-related burst has been observed in other studies examining an even smaller range of saccade amplitudes (Anderson et al. 1998). *In vitro* experiments can be used to determine whether the structure of the intrinsic collicular circuitry and/or the biophysical properties of the output neurons is homogeneous or varies in a manner that could partially account for aspects of the STTP.

Still, the differential weighting of anatomical projections from different parts of the collicular map to the pontine and midbrain regions containing the EBNs must be a critical aspect of the STTP (Grantyn et al. 2002; Moschovakis et al. 1998). Isolated preparations containing the SC and the pontine EBNs or the SC and the midbrain EBNs would allow other aspects of the STTP to be studied directly. Outputs from many regions of the collicular map converge on each EBN (each pontine EBN discharges before movements having a full range of horizontal amplitudes). Thus, in the isolated preparation, intracellular recordings

could be used to examine the efficacy of synaptic inputs arriving from different regions of the collicular map.

The activity of collicular neurons receives extensive inhibitory input from the substantia nigra (SN). Saccades occur when local inhibition is removed by local inactivation of neurons in the SN (Hikosaka and Wurtz 1985b). Isolated preparations containing the SN and SC would allow more detailed descriptions of cellular mechanisms and signal distribution, such that interactions between extrinsic and intrinsic sources of inhibition could be examined.

CONCLUSIONS

The modern era of oculomotor research began with the advent of the chronic microelectrode recording technique in the late 1960s. The ability to correlate the activity of a single neuron with the parameters of an ongoing movement fueled a period of rapid progress. Currently, we are in a new era in which the powerful tools of genetics and molecular biology are being used to seek more mechanistic explanations for the orderly relationships between cell activity and behavior.

Although it is difficult to study episodic movements in slice or dish, important advances in our understanding of the structure and function of the brain areas involved in the generation of episodic movements are occurring at a rapid rate. In this chapter, we highlighted some of the new knowledge that has been gained from studies of tectal slices. The number of studies of the oculomotor system using isolated preparations and/or genetic manipulations is also increasing rapidly. A variety of methods are being used, and studies similar to those described for the SC are being performed in other regions of the oculomotor system. For example, the photolysis of "caged" glutamate is being used in SC slices to study intrinsic connections (Helms et al. 2004). Neurons in the vestibular nucleus that are the postsynaptic targets of floccular Purkinje cells expressing a fluorescent protein were found to possess unique physiological properties (Sekirnjak et al. 2003). *In vivo* intracellular recordings from neurons in an oculomotor neural integrator of the goldfish (e.g., Aksay et al. 2001) are providing valuable new information and insights into how persistent firing rates that reflect the sum of previous excitatory and inhibitory inputs can be implemented. High-density oligonucleotide microarray studies have been used to determine genomic changes in the mouse oculomotor system in response to light deprivation from birth (McMullen et al. 2004). The future yield of experiments using these and other techniques will be high. The cellular and molecular information derived from these studies will spawn experiments that test putative mechanisms in behaving animals, some of which have been altered genetically.

Some of the factors that are likely to impede progress can be identified. Currently, cell and molecular data are obtained from one species and behavioral data from a different species. Fortunately, when viewed from an oculomotor perspective, evolution does not seems to be discontinuous, and the basic function

and structural layout of areas like the SC as well as the pontine and medullary circuits containing the oculomotor neural integrators seem to be similar. Designing appropriate experiments to bridge the molecular and molar experiments is an important task.

The tissue being studied in slice is part of a large distributed network. Network inputs and outputs are removed in the isolated preparation, although for some purposes, this is an advantage. New isolated preparations that permit interactions between different components of the distributed network need to be developed. The potential payoff from such preparations is high.

Noticeably missing from this list are studies using voltage-sensitive dyes or other imaging methods to monitor the simultaneous activity of populations of neurons (cf. Seidemann et al. 2002). Many of the structures involved in the control of eye movements are buried deep in the brain or in the cortical sulci; thus, imaging studies are currently difficult or impossible.

Perhaps the most serious limitation of currently available methods is the inability to study the cellular and molecular mechanisms involved in some of the computations necessary to translate sensory signals into commands for action. Neither chronic preparations nor isolated preparations permit intracellular recordings of the interactions of visual, auditory, and somatosensory stimulation. Stable intracellular recordings are difficult in behaving animals, and the input pathways are not preserved in most isolated preparations. The interaction of sensory signals and corollary discharge copies of motor commands, essential for some known computations, cannot currently be studied in isolated preparations. It will be interesting to see how the inventive neuroscience research community copes with these problems.

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REFERENCES

Aizawa, H., Y. Kobayashi, M. Yamamoto, and T. Isa. 1999. Injection of nicotine into the superior colliculus facilitates occurrence of express saccades in monkeys. *J. Neurophysiol.* **82**:1642–1646.

Aksay, E., G. Gamkrelidze, H.S. Seung, R. Baker, and D.W. Tank. 2001. *In vivo* intracellular recording and perturbation of persistent activity in a neural integrator. *Nat. Neurosci.* **4**:184–193.

Anderson, R.W., E.L. Keller, N.J. Gandhi, and S. Das. 1998. Two-dimensional saccade-related population activity in superior colliculus in monkey. *J. Neurophysiol.* **80**: 798–817.

Behan, M., and P.P. Appell. 1992. Intrinsic circuitry in the cat superior colliculus: Projections from the superficial layers. *J. Comp. Neurol.* **315**:230–243.

Dorris, M.C., M. Paré, and D.P. Munoz. 1997. Neuronal activity in monkey superior colliculus related to the initiation of saccadic eye movements. *J. Neurosci.* **17**: 8566–8579.

Edwards, S.B. 1980. The deep cell layers of the superior colliculus: Their reticular characteristics and structural organization. In: The Reticular Formation Revisited, ed. A. Hobson and M. Brazier, pp. 193–209. New York: Raven.

Endo, T., T. Notomi, R. Shigemoto, and T. Isa. 2003b. Hyperpolarization-activated cation channel and its regulation of dendritic spike initiation in projection neurons of the rat superficial superior colliculus. *Neurosci. Res.* **46(1)**:S145.

Endo, T., Y. Yanagawa, T. Obata, and T. Isa. 2003a. Characteristics of GABAergic neurons in the superficial superior colliculus in mice. *Neurosci. Lett.* **346**:81–84.

Freedman, E.G., and D.L. Sparks. 1997. Activity of cells in the deeper layers of the superior colliculus of rhesus monkey: Evidence for a gaze displacement command. *J. Neurophysiol.* **78**:1669–1690.

Glimcher, P.W., and D.L. Sparks. 1992. Movement selection in advance of action in the superior colliculus. *Nature* **355**:542–545.

Grantyn, A., A.-M. Brandi, D. Dubayle et al. 2002. Density gradients of transsynaptically labeled collicular neurons after injections of rabies virus in the lateral rectus muscle of the rhesus monkey. *J. Comp. Neurol*. **451**:346–361.

Grillner, S. 2003. The motor infrastructure: From ion channels to neuronal networks. *Nat. Rev. Neurosci*. **4**:573–586.

Helms, M.C., G. Ozen, and W.C. Hall. 2004. Organization of the intermediate gray layer of the superior colliculus. I. Intrinsic vertical connections. *J. Neurophysiol.* **91**: 1706–1715.

Higashijima, S., M.A. Masino, G. Mandel, and J.R. Fetcho. 2003. Imaging neuronal activity during zebrafish behavior with a genetically encoded calcium indicator. *J. Neurophysiol.* **90**: 3986–3997.

Hikosaka, O., and R.H. Wurtz. 1985a. Modification of saccadic eye movements by GABA-related substances. I. Effect of muscimol and bicuculline in monkey superior colliculus. *J. Neurophysiol.* **53**:266–291.

Hikosaka, O., and R.H. Wurtz. 1985b. Modification of saccadic eye movements by GABA-related substances. II. Effects of muscimol in monkey substantia nigra pars reticulata. *J. Neurophysiol.* **53**:292–308.

Hilbig, H., and A. Schierwagen. 1994. Interlayer neurones in the rat superior colliculus: A tracer study using Dil/Di-ASP. *Neuroreport* **5**:477–480.

Isa, T. 2002. Intrinsic processing in the mammalian superior colliculus. *Curr. Opin. Neurobiol.* **12**:668–677.

Isa, T., T. Endo, and Y. Saito. 1998a. Nicotinic facilitation of signal transmission in the local circuits of the rat superior colliculus. *Soc. Neurosci. Abstr.* **24**:6013.

Isa, T., T. Endo, and Y. Saito. 1998b. The visuo-motor pathway in the local circuit of the rat superior colliculus. *J. Neurosci.* **15**:8496–8504.

Isa, T, and Y. Saito. 2001. The direct visuo-motor pathway in mammalian superior colliculus: Novel perspective on the interlaminar connection. *Neurosci. Res.* **41**: 107–113.

Jay, M.F., and D.L. Sparks. 1987. Sensorimotor integration in the primate superior colliculus: II. Coordinates of auditory signals. *J. Neurophysiol.* **57**:35–55.

Karabelas, A.B., and A.K. Moschovakis. 1985. Nigral inhibitory termination on efferent neurons of the superior colliculus: An intracellular horseradish peroxidase study in the cat. *J. Comp. Neurol.* **239**:309–329.

Katsuta, H., and T. Isa. 2003. Release from $GABA_A$ receptor-mediated inhibition unmasks interlaminar connection within superior colliculus in anesthetized adult rats. *Neurosci. Res.* **46**:73–83.

Katz, P.S., and R.M. Harris-Warrick. 1999. The evolution of neuronal circuits underlying species-specific behavior. *Curr. Opin. Neurobiol.* **9**:628–633.

Keller, E.L. 1979. Colliculoreticular organization in the oculomotor system. In: Reflex Control of Posture and Movement, vol. 50, ed. R. Granit and O. Pompeiano, pp. 725–734. Amsterdam: Elsevier/North Holland Biomedical Press.

Keller, E.L. 2004. Distributed neural processing in the saccadic system. In: The Oculomotor System: New Approaches for Studying Sensorimotor Integration, ed. W.C. Hall and A.K. Moschovakis, pp. 277–301. Boca Raton: CRC Press.

Lane, R.D., C.A. Bennett-Clarke, D.M. Allan, and R.D. Mooney. 1993. Immunochemical heterogeneity in the tecto-LP pathway of the rat. *J. Comp. Neurol.* **333**:210–222.

Langer, T.P., and R.D. Lund. 1974. The upper layers of the superior colliculus of the rat: A Golgi study. *J. Comp. Neurol.* **158**:418–435.

Lee, P., and W.C. Hall. 1995. Interlaminar connections of the superior colliculus in the tree shrew. II: Projections from the superficial gray to the optic layer. *J. Neurosci.* **12**:573–588.

Lee, P.H., M.C. Helms, G.J. Augustine, and W.C. Hall. 1997. Role of intrinsic synaptic circuitry in collicular sensorimotor integration. *PNAS* **94**:13,299–13,304.

Lee, C., W.H. Rohrer, and D.L. Sparks. 1988. Population coding of saccadic eye movements by neurons in the superior colliculus. *Nature* **332**:357–360.

Lo, F.S., R.J. Cork, and R.R. Mize. 1998. Physiological properties of neurons in the optic layer of the rat's superior colliculus. *J. Neurophysiol.* **80**:331–343.

Ma, T.P., H.W. Cheng, J.A. Czech, and J.A. Rafols. 1990. Intermediate and deep layers of the macaque superior colliculus: A Golgi study. *J. Comp. Neurol.* **295**:92–110.

Maeda, M., T. Shibazaki, and K. Yoshida. 1979. Labyrinthine and visual inputs to the superior colliculus neurons. *Prog. Brain Res.* **50**:735–743.

Marder, E., and L.F. Abbott. 1995. Theory in motion. *Curr. Opin. Neurobiol.* **5**:832–840.

Marder, E., and V. Thirumalai. 2002. Cellular, synaptic, and network effects of neuromodulation. *Neural Netw.* **15**:479–493.

Mays, L.E., and D.L. Sparks. 1980. Dissociation of visual and saccade-related responses in superior colliculus. *J. Neurophysiol.* **43**:207–232.

McMullen, C.A., F.H. Andrade, and J.S. Stahl. 2004. Functional and genomic changes in the mouse ocular motor system in response to light deprivation from birth. *J. Neurosci.* **24**:161–169.

Mize, R.R. 1992. The organization of GABAergic neurons in the mammalian superior colliculus. *Prog. Brain Res.* **90**:219–248.

Mohler, C.W., and R.H. Wurtz. 1976. Organization of monkey superior colliculus. Intermediate layer of monkey superior colliculus: Intermediate layer cells discharging before eye movements. *J. Neurophysiol.* **39**:722–744.

Mooney, R.D., M.M. Nikoletseas, P.R. Hess et al. 1988a. The projection from the superficial to the deep layers of the superior colliculus: An intracellular horseradish peroxidase injection study in the hamster. *J. Neurosci.* **8**:1384–1399.

Mooney, R.D., M.M. Nikoletseas, S.A. Ruiz, and R.W. Rhoades. 1988b. Receptive-field properties and morphological characteristics of the superior collicular neurons that

project to the lateral posterior and dorsal lateral geniculate nuclei in the hamster. *J. Neurophysiol.* **59**:1333–1351.

Moschovakis, A.K., A.B. Karabelas, and S.M. Highstein. 1988. Structure-function relationships in the primate superior colliculus. II. Morphological identity of presaccadic neurons. *J. Neurophysiol.* **60**:263–302.

Moschovakis, A.K., T. Kitama, Y. Dalezios et al. 1998. An anatomical substrate for the spatiotemporal transformation. *J. Neurosci.* **18**:10,219–10,229.

Norita, M. 1980. Neurons and synaptic patterns in the deep layers of the superior colliculus of the cat. A Golgi and electron microscopic study. *J. Comp. Neurol.* **190**:29–48.

Pettit, D.L., M.C. Helms, G.J. Augustine, and W.C. Hall. 1999. Local excitatory circuits in the intermediate gray layer of the superior colliculus. *J. Neurophysiol.* **81**: 1424–1427.

Ritter, D.A., D.H. Bhatt, and J.R. Fetcho. 2001. *In vivo* imaging of zebrafish reveals differences in the spinal networks for escape and swimming movements. *J. Neurosci.* **21**:8956–8965.

Robinson, D.A. 1972. Eye movements evoked by collicular stimulation in the alert monkey. *Vision Res.* **12**:1795–1808.

Saito, Y., and T. Isa. 1999. Electrophysiological and morphological properties of neurons in the rat superior colliculus. I. Neurons in the intermediate layer. *J. Neurophysiol.* **82**:754–767.

Saito, Y., and T. Isa. 2003. Local excitatory network and NMDA receptor activation generate a synchronous and bursting command from the superior colliculus. *J. Neurosci.* **23**:5854–5864.

Saito, Y., and T. Isa. 2004. Laminar specific distribution of lateral excitatory connections in the rat superior colliculus. *J. Neurophysiol.* **92**:3500–3510.

Sakatani, T., and T. Isa. 2004. PC-based high-speed video-oculography for measuring rapid eye movements in mice. *Neurosci. Res.* **49**:123–131.

Schiller, P.H., and M. Stryker. 1972. Single-unit recording and stimulation in superior colliculus of the alert rhesus monkey. *J. Neurophysiol.* **35**:915–924.

Seidemann, E., A. Arieli, A. Grinvald, and H. Slovin. 2002. Dynamics of depolarization and hyperpolarization in the frontal cortex and saccade goal. *Science* **295**:862–865.

Sekirnjak, C., B. Vissel, J. Bollinger, M. Faulstich, and S. du Lac. 2003. Purkinje cell synapses target physiologically unique brainstem neurons. *J. Neurosci.* **23**:6392–6398.

Sooksawate, M., Y. Saito, and T. Isa. 2005. Electrophysiological and morphological properties of identified crossed tecto-reticular neurons in the rat superior colliculus. *Neurosci. Res.* **52**:174–184.

Sparks, D.L. 1978. Functional properties of neurons in the monkey superior colliculus: Coupling of neuronal activity and saccade onset. *Brain Res.* **156**:1–16.

Sparks, D.L. 2002. The brainstem control of saccadic eye movements. *Nat. Rev. Neurosci.* **3**:952–964.

Sparks, D.L. 2004. Commands for coordinated eye and head movements in the primate superior colliculus. In: The Oculomotor System: New Approaches for Studying Sensorimotor Integration, ed. W.C. Hall and A.K. Moschovakis, pp. 303–318. Boca Raton: CRC Press.

Sparks, D.L., and R. Hartwich-Young. 1989. The deeper layers of the superior colliculus. In: Rev. Oculomotor Res., The Neurobiology of Saccadic Eye Movements, ed. R.H. Wurtz and M.E. Goldberg, pp. 213–255. Amsterdam: Elsevier.

Sparks, D.L., C. Lee, and W.H. Rohrer. 1990. Population coding of the direction, amplitude, and velocity of saccadic eye movements by neurons in the superior colliculus. *Cold Spring Harbor Symp. Quant. Biol.*:805–811.

Sparks, D.L., and L.E. Mays. 1980. Movement fields of saccade-related burst neurons in the monkey superior colliculus. *Brain Res.* **190**:39–50.

Sparks, D.L., B. Rohrer, and Y. Zhang. 2000. The role of the superior colliculus in saccade initiation: A study of express saccades and the gap effect. *Vision Res.* **40**:2763–2777.

Sprague, J.M. 1975. Mammalian tectum: Intrinsic organization, afferent inputs, and integrative mechanisms. In: Sensorimotor Function of the Midbrain Tectum, ed. D. Ingle and J.M. Sprague, Neuroscience Res. Prog. Bull. vol. 13, pp. 204–214. Cambridge, MA: MIT Press.

van Alphen, A.M., J.S. Stahl, and C.I. De Zeeuw. 2001. The dynamic characteristics of the mouse horizontal vestibulo-ocular and optokinetic response. *Brain Res.* **890**: 296–305.

Watanabe, M., Y. Kobayashi, Y. Inoue, and T. Isa. 2005. Effects of local nicotinic activation of the superior colliculus on saccades in monkeys. *J. Neurophysiol.* **93**:519–534.

3

Locomotor Microcircuits

A Vertebrate Perspective

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ABSTRACT

Locomotion in vertebrates is produced by neuronal networks intrinsic to the spinal cord, called central pattern generators (CPGs), which can generate rhythmic motor bursts even in the absence of sensory feedback or inputs from the brain. It is necessary for the networks to be sufficiently robust to ensure strict alternation between functionally antagonistic motor neurons over a wide range of locomotor frequencies, but flexible enough to respond adaptively to sensory and descending inputs. The behaviors that these networks produce are overtly different, yet the fundamental cellular and synaptic properties of the underlying networks are apparently quite similar, being governed by common principles of operation.

The basis for network rhythmicity is a set of modules based on an excitatory drive to motor neurons from glutamatergic interneurons, together with reciprocal glycinergic inhibition to ensure that motor neurons innervating antagonistic muscles are active in alternation. CPG networks vary between species and at different stages of development in complexity and modular coupling; however, these differences are largely due to the ways in which shared components (voltage- and ligand-gated ion channels, second messenger-linked receptors, synapses, and neurons) are assembled to accommodate biomechanical constraints and to match behavioral needs. This chapter reviews the composition of spinal locomotor networks and how the components of the network unite to generate species-specific locomotor patterns.

INTRODUCTION

Neurobehavioral Requirements of Networks Underlying Locomotion

One of the most visible and recurring behavioral interactions between any organism and its environment is locomotion. The modes of locomotion employed

by each species are an important determinant of the morphological and physiological adaptations of individual animals. To be effective, a locomotor system must deploy a complex set of synergistic neural and biomechanical components in unison, many of which adhere to common principles of design and operation. In this chapter we focus on rhythmic modes of locomotion, such as walking and swimming, as opposed to the ballistic events that are often used to trigger escape behaviors. We will concentrate on spinal cord networks of vertebrates while recognizing that many principles apply also to invertebrate locomotor systems.

These spinal cord networks have the dedicated role of generating a basic pattern of rhythmic motor activity for axial swimming and, in limbed species, for aboveground locomotion, such as walking, trotting, and galloping. An intense debate of sensory control versus CPG networks as the bases of locomotion continued throughout the first part of the twentieth century, with important contributions from Sherrington, Brown, Gray, and von Holst. The conclusion, reached by detailed studies of spinal and deafferented animals and sensory control mechanisms, was that both sides were right. There is a CPG that provides a detailed motor pattern, which can be effectively adapted to the environment through sensory signals (see Grillner 1985). In the 1980s, the next step could be taken, from establishing the presence of complex CPGs to addressing the detailed intrinsic function of these networks. This became possible with the advent of intracellular recording, cell imaging, and staining techniques, together with new neuropharmacological and molecular techniques. The acquisition of such knowledge, and an understanding of locomotor CPGs, is of fundamental importance not only in itself, but also in the context of rehabilitation from a variety of neurological disorders. Moreover, the cellular and molecular architecture of locomotor CPGs is most likely used in other microcircuits in the brain.

The spinal CPGs that generate efficient and adaptive locomotion must satisfy a set of fundamental requirements. First, the networks underlying locomotion must ensure that the synaptic drive is sufficiently strong to cause the membrane potential of an appropriate number of motor neurons to cross spike threshold. This implies a certain safety factor in the drive to motor neurons. Second, the motor pools controlling antagonistic muscles are usually coupled in anti-phase by reciprocal inhibition (Figure 3.1); more complex patterns, conserved between many species, are also generated in some muscles. Third, the detailed behavioral requirements of locomotion are prone to change in multiple time domains, from the need to alter movements rapidly in response to novel sensory inputs, to the often protracted modifications that accompany developmental growth and maturation or injury. As a result, CPGs must express a degree of both short-term flexibility and longer-term plasticity to be able to adapt their output in response to novel behavioral, developmental, and pathological circumstances.

Given the complexity of the adult mammalian spinal cord, there has been a need for "simpler" vertebrate model systems in which a cellular approach is

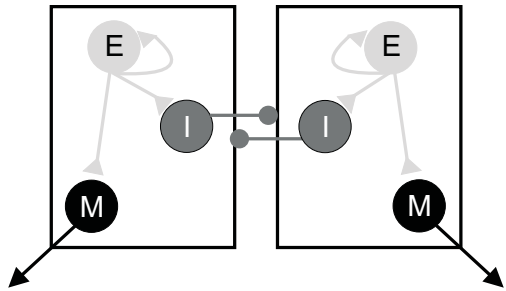

Figure 3.1 The basic organization of a locomotor network module comprising half-center oscillators (boxes) connected by reciprocally inhibitory synapses (dark gray circles) from inhibitory interneurons (I). Within a half-center, excitation is from glutamatergic interneurons (E), which synapse (triangles) with motor neurons (M), inhibitory interneurons, and themselves. This organization is similar to that found in the simple spinal network of the *Xenopus* frog embryo (for a review, see Roberts et al. 1997). Note: circles represent populations of neurons.

more feasible. The lamprey (cyclostome) brainstem–spinal cord provides one particularly well-studied adult model (Grillner 2003; Figure 3.2) that may reveal the ancient vertebrate locomotor pattern before it became more complicated through the addition of paired fins and limbs. Comparisons of the lamprey network (Grillner 2003) with that controlling swimming in frog embryos (Roberts et al. 1997) have revealed many remarkable similarities, suggesting common principles of operation in locomotor networks. Frog embryos, zebrafish larvae, and mammalian neonates are also important models, which additionally provide information on how networks are formed during development (for a collection of recent reviews from different model systems, see McClellan 2000). The ontogenetic origins of cellular and network rhythmicity are important not only in their own right, but also as a tool to understand how and why the complex networks of adult vertebrates evolved and function in the ways that they do. Some general principles have emerged. First, the networks become functional remarkably early in development, often ahead of the behaviors that they will eventually control. Second, movements produced by embryonic networks lack the precision and flexibility displayed by adults. Third, the maturation of locomotor activity is associated with the developmental integration of descending modulatory inputs, many of which coincide approximately with birth or hatching. These extrinsic influences have been shown in some cases to be important or even causal to the maturation process. To a quite remarkable extent, certain basic principles of network operation have been discovered at the center of overtly different locomotor CPGs. Taken together, the evidence indicates that an ancestral or "core" network forms the basis for generating the segmental alternating rhythm for swimming in lamprey, fish, or tadpole and that with further development or evolution, layers of complexity have been bolted onto this elementary

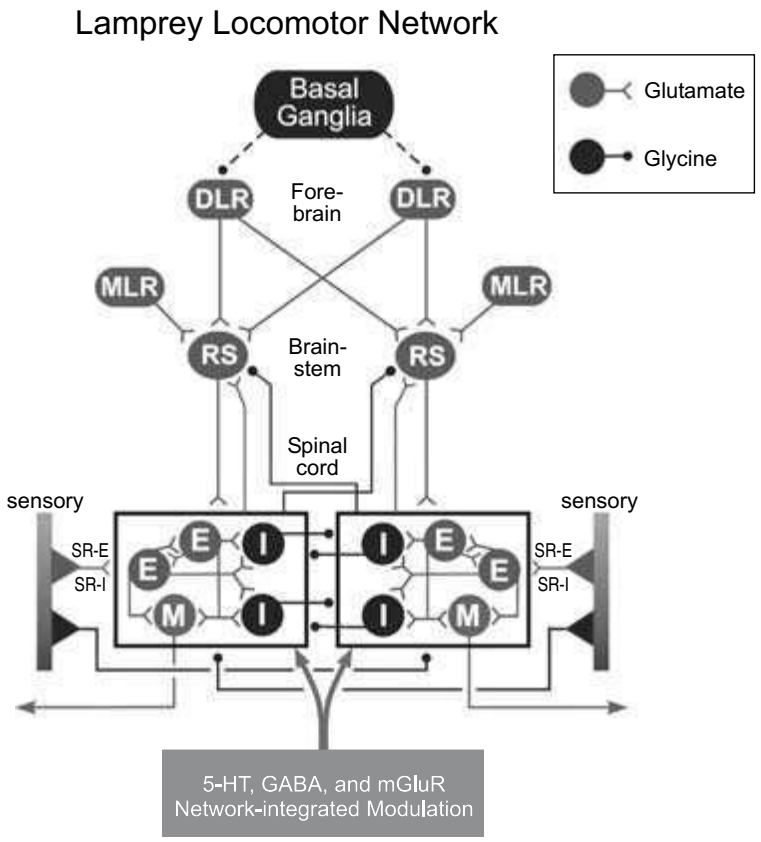

Figure 3.2 Elaboration of the locomotor network in the lamprey. Schematic representation of the forebrain, brainstem, and spinal components of the core neural circuitry that generates rhythmic locomotor activity. All neuron symbols denote populations rather than single cells. The reticulospinal (RS) glutamatergic neurons excite all classes of spinal interneurons and motor neurons. The excitatory interneurons (E) excite all types of spinal neurons: the inhibitory glycinergic interneurons (I), which cross the midline to inhibit all neuron types on the contralateral side, and motor neurons (M). The stretch receptor neurons are of two types: excitatory (SR-E), which excite ipsilateral neurons, and inhibitory (SR-I), which cross the midline to inhibit contralateral neurons. RS neurons receive excitatory synaptic input from the diencephalic and mesopontine locomotor regions (DLR and MLR, respectively), which receive input from the basal ganglia as well as visual and olfactory input. Metabotropic receptors are also activated during locomotion and are an integral part of the network. 5-HT: 5-hydroxytryptamine serotonin; GABA: γ-aminobutyric acid; mGluR: metabotropic glutamate receptor; dashed lines indicate indirect connections.

rhythm-generating module (Fetcho 1991; Kiehn et al. 1997; Kiehn and Kullander 2004). Thus, the locomotor networks of adult mammals may have evolved through the progressive elaboration and duplication of an ancestral

segmental network appropriate for swimming via intermediates requiring the control of fins on the two sides and then segmentally restricted networks for the control of multi-jointed limbs. That these systems have a common origin is also supported by the fact that the brainstem locomotor areas that control the level of locomotor activity are conserved from lamprey to primates. The spinal locomotor and brainstem respiratory networks appear to have much in common in terms of membrane properties and neurotransmitters, although the respiratory network itself remains to be completely elucidated. It is interesting to note that these two oscillatory systems can indeed be coupled (Morin and Viala 2002).

THE COMPONENTS OF THE NETWORK

The CPGs for locomotion, whether walking or swimming, are located primarily in the spinal cord, but may extend into the hindbrain. Activity in the CPG can be initiated and maintained by descending reticulospinal fibers from the brainstem. The reticulospinal neurons are, in turn, controlled from the mesencephalic and diencephalic locomotor region, both of which have been conserved throughout evolution. (Figure 3.2; Orlovsky et al. 1999). The forebrain and brainstem control of locomotion was outside the scope of this Dahlem Workshop and will thus not be discussed further. To understand how a given CPG is able to satisfy the basic requirements of locomotion, it is important to ask: What are the components or "building blocks" of the network? The core of the segmental CPG consists of pools of excitatory (glutamatergic) interneurons, which excite each other, and crossed glycinergic interneurons, which generate the left–right alternating pattern. They provide the input to the segmental motor neurons (Figures 3.1, 3.2). Motor neurons and premotor interneurons (Figure 3.1) are each endowed with a particular set of ion channels and receptors and are wired together by a complex anatomical mesh of synaptic connections. Before describing the principles of operation at the network level, we first review briefly how the voltage- and ligand-gated ion channels contribute to the pattern generation.

Ion Channels of Particular Importance for the Operation of the Locomotor Central Pattern Generators

The behavior of a given neuron is determined by the combination of ion channels expressed in the cell membrane and by transmitter-activated receptors. Below we will highlight a few ion channels that are of critical importance from the perspective of generating the rhythmic activity within a pool of excitatory interneurons. The examples are taken mainly from the lamprey (Figure 3.3; Grillner et al. 2001; Grillner 2003), but many are expressed in other species, such as the frog embryo (Dale and Kuenzi 1997), where similar functions are performed.

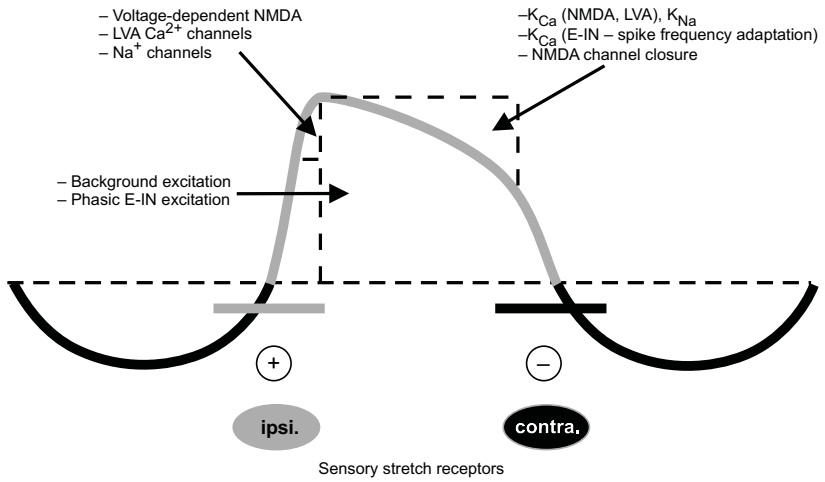

Figure 3.3 Factors controlling burst onset and termination. Several factors contribute to the initiation, maintenance, and termination of the depolarizing phase. In addition to conventional synaptic, voltage-dependent NMDA (*N*-methyl-$_D$-aspartate) receptors, low voltage-activated Ca^{2+} channels (LVA-Ca^{2+}) and Na^{+} channels might be activated. Ca^{2+} enters the cell through these channels, activates K_{Ca} channels, and initiates a progressive hyperpolarization leading to closure of the NMDA channels. The initiation of depolarization is facilitated by activation of ipsilateral excitatory stretch receptors, whereas its termination is partially a result of activation of contralateral inhibitory stretch receptors. E-IN: excitatory interneuron; dashed line indicates the resting membrane potential.

Spike Frequency Regulation: Role of Afterhyperpolarization

The regulation of spike frequency during locomotion is one factor of paramount importance. It has two components: the absolute frequency produced by a given excitatory drive and the spike frequency adaptation within a burst. Usually the first few inter-spike intervals upon a tonic excitation are shorter than subsequent ones. The main determinant is the slow afterhyperpolarization (sAHP) following each action potential. The sAHP is mainly due to calcium-dependent potassium channels (K_{Ca}, SK3-type) activated by the calcium entering the cell through N-type calcium channels during the action potential. At higher frequencies, sodium-activated potassium channels are also of importance. They are activated by the accumulated sodium entry during successive action potentials.

Control of Action Potential Duration: Role of K^{+} Channel Subtypes

The duration of the action potential is finely controlled, which is important since this determines the amount of calcium entry, which in turn activates K_{Ca} channels and determines the amplitude and duration of the afterhyperpolarization.

Voltage-dependent sodium channels are responsible for the upstroke of the action potential, whereas several subtypes of voltage-dependent potassium channels ensure that the duration of the action potential is kept constant. High threshold K_v 3.4 and another subtype of sodium-activated potassium channels are the main determinants of action potential duration, but delayed rectifiers can also contribute, particularly if the action potential duration is prolonged (e.g., when the former types of K^+ channels are blocked by selective antagonists). Several different modulators can effect the action potential duration and thereby either (a) reduce calcium entry, as occurs both at the presynaptic level leading to presynaptic inhibition or at the somatodendritic level, when the sAHP is reduced (due to K_{Ca}), or (b) enhance calcium entry and thereby produce the converse effects. Aminergic and peptidergic receptors serve as powerful modulators of various calcium channel subtypes.

Processes that Boost the Membrane Depolarization

As the membrane potential recovers from being inhibited and enters the depolarizing phase, there are several factors in addition to the synaptic excitation that may boost the membrane depolarization. One important factor is the activation of low voltage-activated calcium channels (T-type), which will enhance the depolarization at levels close to the threshold for the action potential. At depolarized but subthreshold membrane potentials, voltage-dependent sodium channels located at the dendritic level may also provide a further depolarization (Hu et al. 2002). Finally, voltage-dependent NMDA channels also open up close to threshold. These factors contribute significantly to ascertain that the neurons are effectively depolarized.

The Depolarizing Plateau

A neuron that fires action potentials during a burst will obviously be affected by the sAHP summation (see above). In addition, the calcium entry occurring through a variety of sources will produce further activation of K_{Ca} channels, which will tend to hyperpolarize the membrane potential. The activation of voltage-dependent NMDA channels can also promote the development of a plateau depolarization, which helps maintain a continuous membrane potential depolarization. An additional factor that is known to play a role at the brainstem level is a calcium-activated cation channel (I_{can}). These channels may contribute also during slow locomotor activity involving very long-lasting motor bursts.

The Termination of the Depolarizing Phase

Progressive activation of K_{Ca} channels help terminate the depolarizing phase of activity. The decrease in firing activity within the pool of excitatory

interneurons in itself leads to a reduced excitatory drive. The voltage dependence of NMDA channels leads to a progressive closure of these channels due to the gradual hyperpolarization that will ensue. This leads to the regenerative hyperpolarization that, together with the onset of reciprocal inhibition, returns the membrane potential to the inhibitory phase of the cycle of activity. The blockade of K_{Ca} channels causes a breakdown of locomotor activity, particularly during slow locomotor activity. All types of Ca^{2+} channels except L-channels appear to be of importance for pattern generation, and an interference with the K_V 3.4 channel produces severe interference.

COMMON PRINCIPLES OF LOCOMOTOR NETWORK OPERATION

Now that we have considered some of the important biophysical properties of neurons within locomotor networks and the underlying ion channels, let us return to the way in which these neurons are synaptically coupled to generate coordinated rhythmic activity. The basic locomotor network module utilizes glutamatergic excitation within each half-center, with pairs of half-centers coupled by reciprocal glycinergic inhibition (Figures 3.1, 3.2). Each half-center can generate burst activity by itself without inhibition, but the crossed inhibition ensures the alternating pattern between antagonists. This modular structure applies to both the coupling of segmental "unit burst generators" on the left and right sides of the spinal cord of an axially based system (Figure 3.4A) as well as to the antagonistic muscles operating the joints of a limb on the same side (Figure 3.4B; see Grillner 1985). In both cases, the serially iterated modules are coupled appropriately to neighboring modules. For example, modules on opposite sides of the cord in the limbed system must be coupled by crossed excitatory and inhibitory pathways (Figure 3.4B) to allow for changes in gait, for example, from walking to hopping (Kiehn and Kullander 2004). The motor pattern of a single limb can be reduced, to a first approximation, to alternating activity in each group of close antagonists of a given joint; however, some motor neuronal groups are active primarily in the interval between flexors and extensors (see Grillner 1985).

Role and Sources of Inhibition

The strength of the evidence for the network connectivity of the core module shared by these different locomotor systems varies among preparations. The case for glycinergic reciprocal inhibition is the most comprehensive and will thus be reviewed first. The reciprocal inhibition between antagonistic motor pools on opposite sides of the spinal cord is chloride ion-dependent and glycinergic (for references, see Grillner 2003; Roberts et al. 1997; Kiehn et al.

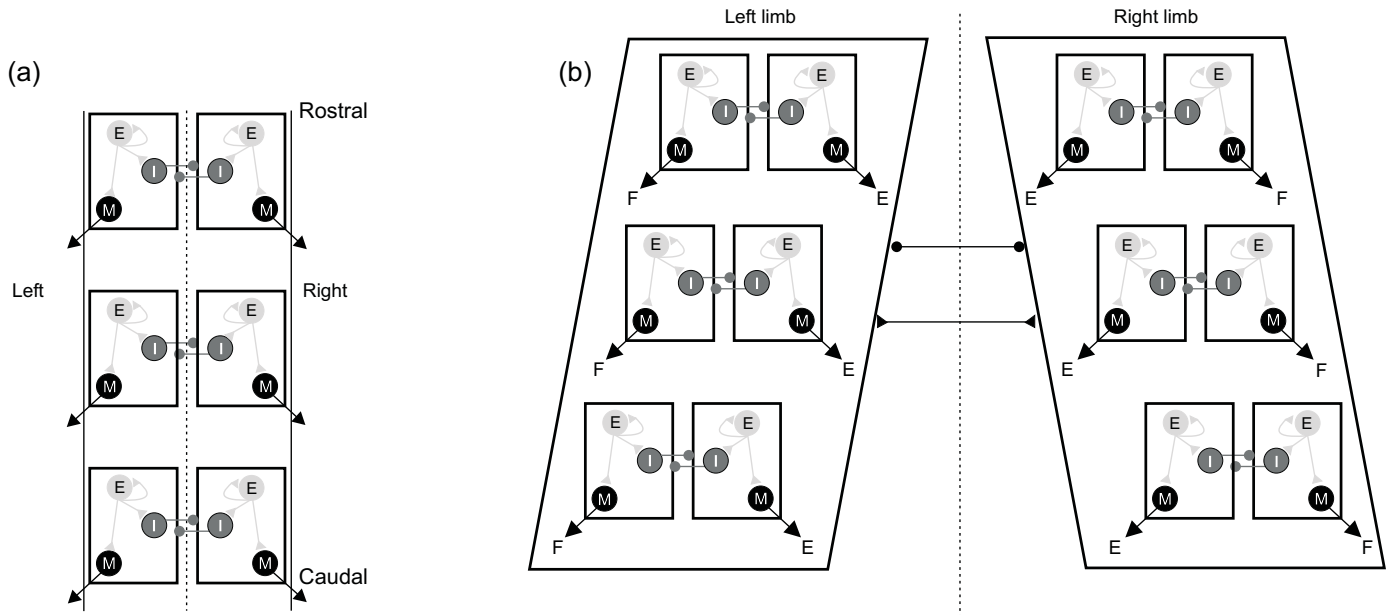

Figure 3.4 The organization of rhythm-generating modules in different types of locomotor central pattern generator. (a) In axially based swimming systems, like the lamprey, modules are distributed along the rostrocaudal neuroaxis and are coupled so as to generate a head-to-tail wave of activity, which alternates between the two sides. (b) In limbed systems, sets of modules that function as unit burst generators for the different joints of a limb are present on the left and right sides and may be coupled by both crossed inhibition and crossed excitation to allow for gait changes. Dashed line represents midline. I: inhibitory neurons; E: excitatory neurons; M: motor neurons.

1997). Moreover, immunocytochemical studies have revealed the presence of glycine-positive neurons with commissural projections that are rhythmically active in time with their half-center of origin and are therefore presumed to be responsible for mid-cycle inhibitory postsynaptic potentials (IPSPs) in motor neurons during tadpole locomotor network activity (Dale et al. 1986). Thus, the primary role of reciprocal inhibition is to ensure that antagonistic locomotor network neurons are active in alternation. This, however, is not the only way in which IPSPs contribute to rhythm generation; the hyperpolarization resulting from inhibition modifies the integrative electrical properties of motor and premotor interneurons. For example, hyperpolarization helps remove any residual fast Na^+ channel inactivation that has accumulated during the preceding cycle and promotes K^+ channel deactivation. This will have a restorative function and lead to an increase in the excitability of neurons coincident with the onset of the next cycle. At the same time, the inhibition will turn on any hyperpolarization-activated conductances, such as Ih, which, in turn, will increase the probability of rebound firing, once again priming the network for the next cycle. The same applies for low voltage-activated Ca^{2+} channels that provide postinhibitory rebound depolarization close to spike threshold. Since the extent of Na^+ channel inactivation and Ih activation are both voltage dependent, changes in the amplitude of the reciprocal inhibition have a significant effect on the network as a whole. Indeed pharmacological, anatomical lesioning, and computer simulation studies all indicate that the strength of these connections is important in setting the cycle period of locomotion. Increasing the strength of reciprocal inhibition reduces the cycle frequency (and vice versa), and this feature of glycinergic connections renders them a key target for neuromodulation (Dale 1995; Hellgren et al. 1992; Sillar et al. 2002; Cangiano and Grillner 2003).

In general, the inhibitory input to motor neurons is purely glycinergic with regard to the CPG and spinal reflexes (cf. Jonas et al. 1998 and below). There is, however, an extensive GABAergic innervation in the dorsal horn and other parts of the gray matter. A powerful presynaptic, phase-dependent modulation of both sensory terminals, and inhibitory and excitatory premotor interneurons occurs during CPG activity. This modulation is driven by GABA interneurons activating both $GABA_A$ and $GABA_B$ receptors. A postsynaptic $GABA_B$ modulation of different Ca^{2+} channel subtypes also occurs (reviewed in Grillner 2003). The GABA action during ongoing locomotion causes a very significant reduction of the locomotor burst rate, by up to 50%.

Some spinal neurons appear to store either GABA or glycine in vesicles, whereas others store both. During development there appears to be a gradual shift from GABA to glycine in some neurons. In neonatal mammals GABA is thus co-released from some spinal glycinergic interneurons (Jonas et al. 1998). Postsynaptic glycine receptors distinguish themselves from most other ionotropic receptors in being bereft (so far as is known) of modulatory sites. In contrast, $GABA_A$ receptors are notable for the plethora of sites for endogenous

(e.g., neurosteroids) and pharmaceutically targeted (e.g., benzodiazapines, barbiturates, general anesthetic) binding sites. Thus, benzodiazepines cause a marked slowing of the locomotor burst rate. The crossed inhibitory neurons studied in the locomotor system (lamprey, tadpole) are blocked by strychnine and are thus glycinergic; they are, however, subject to GABAergic presynaptic inhibition.

Role and Sources of Excitation

The pool of segmental excitatory interneurons is glutamatergic and activates both AMPA and NMDA receptors (Dale and Roberts 1985; Buchanan and Grillner 1988) and also metabotropic glutamate receptors (mGluR1, mGluR5; El Manira et al. 2000). Interneurons that are presumed to be excitatory in the neonatal mammalian spinal cord have also been shown to be immunopositive for glutamate transporters (Kullander et al. 2003). The function of glutamate receptor activation is primarily to drive motor neurons above spike threshold during the depolarized phase of each locomotor cycle. However, activation of the NMDA subtype of receptor (which occurs simultaneously with AMPA receptor activation) also has important subsidiary roles that are directly relevant to rhythm generation. In the *Xenopus* embryo, for instance, NMDA receptor-mediated EPSPs are long duration (ca. 200 ms) compared to the swimming cycle periods (ca. 50–100 ms) and so summate from one cycle to the next to produce tonic excitatory drive that is important for the maintenance of activity (Dale and Roberts 1985). Another important feature of NMDA receptor activation is that it leads to the induction of depolarizing plateaus or oscillatory membrane properties that contribute to the drive, either in each cycle or over many consecutive cycles (see below).

In addition to glutamate, there are two complementary sources of excitation that are known to contribute in some systems or stages of development:

1. *Acetylcholine*. Convincing evidence from the *Xenopus* embryo has demonstrated that motor neurons make central cholinergic connections onto each other and also onto premotor rhythm-generating interneurons, which contributes to the synaptic drive for swimming (Perrins and Roberts 1995). There is also some evidence from other preparations, although acetylcholine receptors are known to be present on motor neurons (see Grillner 2003).
2. *Electrical coupling.* Motor neurons have been shown to be electrically coupled, and this coupling may play an important role in the timing of motor activity, not only at immature stages, such as *Xenopus* (Perrins and Roberts 1995) and zebrafish (Drapeau et al. 2002) embryos, where gap junctions are generally more prevalent, but also in the adult (Kiehn and Tresch 2002). The function of the electrical coupling in spinal motor networks, as in other microcircuits, is to enhance the synchronization of

neuronal activity. The coupling in *Xenopus* embryos is restricted to neurons located within a few cell bodies of each other (Perrins and Roberts 1995; Roberts et al. 1997), so it is possible that these are homonymous motor neurons innervating a given segmental muscle block. In the neonatal rat, spinal cord gap junctions contribute to rhythm generation and help to coordinate the oscillatory membrane behavior of neurons even in the absence of action potential firing. Thus, application of NMDA and 5-HT produces stable rhythmic locomotor-like impulse activity, but when tetrodotoxin is applied, oscillations in membrane potential remain. These oscillations derive from two sources: one is intrinsic to the recorded neuron and the other reflects the inputs filtering through gap junctions from neighboring coupled cells in the spinal network, which are also oscillating but at a different frequency. This network can be decoupled by applying the gap junction blocker, carbenoxolone.

A variety of gap junction blockers, like carbenoxolone, have been shown to affect circuits in different parts of the nervous system. A major problem is that these blockers are fairly nonspecific and can affect a variety of channels. Thus it is difficult to use them in a conclusive matter, unless one has good control over both the pre- and postsynaptic sites, which is, however, usually not the case. One area of research that has yet to be explored, especially given this role of gap junctions in the synchronization of neuronal activity and the more extensive electrical coupling in networks at earlier stages, is the role that gap junctions and their modulation might play in the maturation and intrinsic functioning of locomotor networks.

INTRINSIC FUNCTIONING OF THE NETWORK

There are circumstances under which one might imagine it being advantageous for locomotion to be semi-autonomous and self-sustaining; for example, during prey pursuit and predator evasion or during migration in certain birds and fish. There is now substantial evidence from many different model systems that the central networks themselves possess properties to allow co-coordinated rhythmic activity to continue unabated for many hundreds of consecutive cycles, even in the complete absence of sensory feedback. There are, however, two complementary ways in which this network function can be regulated. One involves emergent properties of network design, which reflect ways in which the neuronal microcircuits are constructed, as described below. Another is the process of intrinsic neuromodulation in which certain signaling molecules, though not required for basic rhythm generation, are released during activity to modify one or more parameters of locomotor activity.

Several important features of motor microcircuit design contribute not only to the generation and maintenance of the locomotor rhythm, but also to how it adjusts the motor output in response to external contingencies. These include

positive feedback excitation (where the excitatory interneurons feed back synaptic excitation within their own population), cellular properties of network interneurons, activity-dependent synaptic modulation (where the strength of a synaptic connection alters with some parameter of the output, such as rhythm frequency), intrinsic oscillatory membrane properties (in which neurons are endowed with cellular properties that generate or contribute to rhythmicity), and feedforward inhibition (where the direct excitatory drive to motor neurons is paralleled by a delayed disynaptic inhibitory input).

Positive Feedback Excitation

Interneurons and motor neurons alike receive very similar EAA receptor-mediated synaptic drive (AMPA and NMDA) during motor activity. One possible way in which burst activity in a network sustains itself is that the excitatory interneurons make synaptic connections, not just with motor neurons but among each other (Parker and Grillner 2000; Roberts et al. 1997). In this way the excitation occurring during a burst can be maintained at a level sufficient for rhythm generation. However, direct evidence for this is limited, presumably because of the technical difficulties of recording from two identified excitatory interneurons that contact each other, and it has never been shown directly that a given CPG interneuron excites itself. Such connections may be rare, however, since absolute levels of excitation must be kept in balance. Computer simulations of locomotor networks support the presumption that positive feedback excitation exists, because the omission of mutual excitatory connections can prevent sustained rhythmic activity. The positive feedback excitation can thus maintain burst activity. Towards the end of the burst, cellular properties (see above) reduce the likelihood that neurons will discharge, leading to a situation in which a number of factors combine to terminate the burst in a somewhat "regenerative" fashion—another type of positive feedback mechanism.

Ipsilateral Inhibitory Neurons Are Not Required for Burst Termination

A key parameter of any network generating locomotion is the duration and intensity of firing of motor neurons within a given cycle, that is, the motor burst structure. Several network cellular properties converge to determine this property, which in turn governs the force and duration of contraction in the target muscle. In the lamprey, rhythmic motor neuron bursting can be generated in the ipsilateral spinal cord after a longitudinal midline section, even after a blockade of inhibitory synaptic transmission (Cangiano and Grillner 2003). Moreover, a strychnine blockade does not affect the burst rate of the ipsilateral segmental spinal cord burst generators, whether at a high or a low burst rate. The crossed inhibitory synaptic effects determine the right–left alternation and, in addition slow down the burst rate by an inhibitory effect on the unilateral CPG. In

addition to inhibitory interneurons with contralateral axons, other interneurons that target monosynaptically both ipsilateral motor neurons and crossed inhibitory interneurons are also present. They both receive locomotor-related excitatory modulation from the ipsilateral unit CPG in phase with the motor neurons. These ipsilateral inhibitory interneurons are of two types: the large lateral interneurons present in the rostral part of the spinal cord that have long descending axons, and the small inhibitory interneurons distributed along the spinal cord (Buchanan and Grillner 1988). A proportion of the latter can be activated monosynaptically by excitatory interneurons (Parker 2003). Both types of ipsilateral interneurons are thus depolarized during the ipsilateral burst and could, if made to spike during mid-burst, contribute to burst termination provided that the burst duration is relatively brief (Hellgren et al. 1992). These interneurons are, however, apparently not required for burst generation to occur (see above), but they could nevertheless act as a complementary factor in burst control. During steering, on the other hand, a strong synchronous activation of both these interneurons could effectively serve to terminate the crossed inhibition and thereby help to initiate a contralateral burst and a turning movement to the contralateral side (see Grillner 2003).

Activity-dependent Synaptic Modification

The strength of synaptic connections within locomotor networks is subject to continuous modification. Facilitating synapses activated by a brief presynaptic train will become progressively more effective, while depressing synapses become weaker. The postsynaptic response thus varies with presynaptic firing history. In the lamprey, the reciprocal inhibitory synapses are of the depressing type, while many of the excitatory synapses are facilitating. Moreover, this activity-dependent modulation is in itself subject to a powerful modulation by 5-HT and substance P (Parker and Grillner 2000). For instance, 5-HT modifies the crossed inhibitory synapse from a depressing to a facilitating synapse, an effect that contributes to the 5-HT-induced slowing of the burst rate (Kozlov et al. 2001). The synaptic connections are thus self-tuning in a manner that will impact on integration within the network in a frequency-dependent fashion.

Intrinsic Oscillatory Membrane Properties

Selected conductances can unite to endow motor neurons with voltage-dependent bi-stable or oscillatory membrane properties, which in vertebrates at least are conditional rather than endogenous. These properties bestow on motor neurons the capability to regulate dynamically their own output, in addition to responding to phasic synaptic drive. In the spinal motor networks of both lower and higher vertebrates alike, intrinsic oscillations in the membrane potential of motor neurons are conditional upon the activation of NMDA receptors. The

underlying mechanism, first described for lamprey spinal motor neurons (reviewed in Grillner et al. 2001; Grillner 2003), relies upon the voltage dependence of the NMDA receptor ionophore in the presence of Mg^{2+} ions. Similar oscillations have now been described in other locomotor networks, including those in mammals, and they appear to contribute to network output from early in the development of the networks (McLean et al. 2000). In amphibian tadpoles and in neonatal rats, NMDA receptor-mediated oscillations are also present, but they appear to differ from those in the lamprey by being conditional upon the coactivation of 5-HT and NMDA receptors. One possible explanation for this difference could be that there is sufficient 5-HT present in the isolated adult lamprey spinal cord to gate the oscillations and there is a logical intrinsic source in the ventromedial plexus. The bath application of NMDA could activate NMDA receptors on these ventromedial plexus cells causing them to depolarize and release their modulatory contents. Conversely, the reliance of the oscillations on 5-HT in the tadpole and the neonatal rat may be related to the developmental immaturity of these locomotor systems.

The manner in which oscillatory membrane properties are deployed during locomotion is less well understood. In the lamprey spinal cord there is evidence that they contribute to the underlying voltage fluctuations that occur within each cycle of fictive swimming. The rising phase of the oscillation would naturally assist in bringing the motor neurons above threshold, the plateau phase regulates intra-burst firing frequency, and the falling phase is a mechanism that contributes to the termination of the burst. Whether or not the conductances occurring in the trough of the intrinsic oscillatory cycle function as an inhibitory shunt in the inter-burst period has not yet been demonstrated, but this seems likely. In addition, the frequency of the oscillations is rather slow suggesting that they are unlikely to contribute across the full range of swimming frequencies. In amphibian tadpoles this disparity between the frequency of the intrinsic oscillations (ca. 0.5 Hz) and the swimming frequency (10 to 30 Hz) is even more acute. In this system, however, there is evidence that the oscillations may contribute to the modulation of swimming frequency and intensity over tens of cycles. Thus, a brief 5-HT input could trigger one or more oscillations that cause the acceleration of swimming (during the depolarizing phase), which merges with the synaptic and network activity, resulting in a harmonic modulation of locomotor behavior.

INTRINSIC NEUROMODULATION: REGULATION OF THE NETWORK BY THE NETWORK

The final output of any neuronal network depends upon the interplay between synaptic inputs and the integrative electrical properties of neurons embedded in that network. As described above, motor neurons and interneurons are endowed

with a complex array of ion channels that determine collectively how these cells will respond at any given moment in time. Moreover, in many cases this basic set of conductances is subject to complex neuromodulation both from within the network (see below) and through extrinsic inputs (Pflüger and Büschges, this volume).

The term "intrinsic neuromodulation" refers to the regulation of network activity by substances released from within the network during locomotor activity (Kiehn and Katz 1999). In general, these neuromodulators activate metabotropic receptors to influence either ion channels or the integrative electrical properties of component neurons or the strength of synaptic connections within the network, or both. Most is known about the effects of amino acids (GABA, glutamate) and amines, especially 5-HT and the peptide substance P. The activation of their target receptors leads to modulation of one or more aspects of the motor rhythm. Some modulators affect preferentially particular facets of the rhythm, while others exert more global effects. It is important to note that there will almost certainly be a convergence of neuromodulatory effects from potentially all of these neuromodulators simultaneously, which will merge to sculpt the final motor output pattern.

Role of Metabotropic Glutamate Receptors

Homosynaptic modulation, in which synaptic connections are self-regulating, usually results from the release of transmitter, which activates presynaptic metabotropic autoreceptors to produce an activity-dependent negative feedback control of transmitter release. One example of this in relation to spinal motor networks involves presynaptic activation of metabotropic glutamate receptors (mGluRs). In the lamprey spinal cord excitatory transmission is depressed following activation of certain subtypes of presynaptic metabotropic glutamate receptors (mGluR II, III) (El Manira et al. 2000).

In addition to the postsynaptic activation of ionotropic AMPA and NMDA receptors postsynaptic mGluR I (mGluR1 and 5 subtypes) receptors are also activated, leading to an enhanced excitation via a gamut of effects including the potentiation of currents flowing through NMDA receptor ion channels and by the inhibition of a leak K^+ current. This latter effect leads to membrane potential depolarization in concert with a decrease in membrane conductance, thus rendering the postsynaptic neurons more receptive to excitatory inputs. In addition, mGluR1 interacts with endocannabinoids, which contribute to the enhanced excitation in part through a reduction in reciprocal inhibition (El Manira, pers. comm; for a review, see Grillner 2003). In combination, these different mechanisms, which are engaged following glutamate release, will regulate the strength of excitatory synaptic connections such that they are inherently predisposed to function within set limits of gain.

5-HT Modulation of the Locomotor Network

It appears that in practically all vertebrate locomotor CPGs, 5-HT has a profound modulatory effect on locomotor burst activity. In the lamprey the intraspinal 5-HT system is turned on during each period of locomotor activity. The net effect is also a slowing of the burst rate. 5-HT exerts several well-studied effects on both the somatodendritic and the presynaptic level (see Grillner 2003).

GABAergic Modulation of Network Activity

In vertebrates, from lamprey to mammals, a GABAergic modulation occurs that modulates network neurons at both the pre- and postsynaptic levels via both $GABA_A$ and $GABA_B$ receptors (see also above). The net result of the GABAergic action is a marked slowing of the burst rate. The rationale (if any) for this consistent modulation may be an optimization of the properties of network neurons and synapses to provide a stable burst rate.

Modulation of the Duration of Locomotor Episodes by GABA and Purines

Activity-dependent modulation can also occur heterosynaptically. In this case, the activity of one member of the network influences the interactions between other members of the same network. There are many examples of this, and each has a particular impact on the network. One intriguing example relates to the termination of locomotor episodes; obviously, locomotion has intricate mechanisms to ensure that it is self-sustaining, as described above, but *eventually* it must terminate. In the *Xenopus* tadpole, two quite different mechanisms have been discovered. The first is conventional in the sense that it involves activation of GABAergic neurons extrinsic to the spinal cord, located in the hindbrain, which in turn inhibit swimming by activating $GABA_A$ receptors on spinal neurons to terminate prematurely bouts of locomotor activity (Roberts et al. 1997; McLean et al. 2000). However, intrinsic to the spinal network itself there is an alternative mechanism involving the release of adenosine triphosphate (ATP) during swimming activity (Dale and Gilday 1996). Although it is not yet known which member(s) of the network are the sources of the ATP, its initial effect is to block K^+ channels, thereby raising excitability and ensuring rapid swimming at the onset of an episode. ATP is then broken down, with a delay, by extracellular endonucleotidase enzymes to adenosine, which in turn blocks calcium channels and hence reduces synaptic transmission to inhibit neural activity. Thus, during the course of an episode of swimming, the initial excitatory effect of a high level of ATP gradually diminishes as it is converted into a bi-product that has the opposite, inhibitory effect. At some critical stage in this biochemical process adenosine levels become dominant and swimming ceases.

Long-term Modulation of Locomotor Burst Frequency

A form of modulation with the opposite effect, whereby the excitability of the network is enhanced for long periods, is also possible, and an example of this involves neuropeptides of the tachykinin family (Parker and Grillner 1999; reviewed in Grillner 2003). When substance P is applied for a brief period to the lamprey spinal cord, it triggers both short- and long-term changes in NMDA-induced swimming. The short-term effects are mediated by a variety of second messenger-mediated changes in ion channels and NMDA receptors, whereas the long-lasting effects, which require an elevated cytosolic Ca^{2+}, involve the induction of new protein synthesis by substance P (Parker and Grillner 1999). In both cases there is an increase in the excitability of the network and a decrease in the cycle-by-cycle variability in motor burst structure; as a result, swimming becomes faster and more regular. Thus, substance P is capable of inducing plastic changes in the function of the locomotor network. Although there are potential sources of substance P that could conceivably be responsible for an endogenous plasticity of this type, there is no information on the behavioral circumstances under which the plasticity is normally deployed. One possibility is that during the long migrations upon which the lampreys can embark, activity-dependent release of substance P leads to a positive feedback reinforcement of locomotor activity. Such a mechanism for long-term plasticity in locomotor networks could also potentially provide a plausible explanation for other locomotor-related phenomena, such as the effects of training in sports endurance events or during rehabilitation from spinal cord injury.

CONCLUSIONS

Steps in the Production of Locomotor Rhythmicity

We can now summarize the key cellular and synaptic steps in the generation of rhythmic motor neuron bursts during a cycle of locomotor activity (Figure 3.5). At the start of a cycle (1), activation of AMPA-type glutamate receptors depolarizes the membrane potential towards spike threshold and into the voltage region where the block of NMDA receptor ion channels by Mg^{2+} ions is alleviated. Firing begins as the membrane potential depolarization, driven partly by oscillatory membrane properties, accelerates into the burst (2).

The activation of K_{Ca} channels by calcium entry through both voltage-dependent calcium channels and NMDA channels causes spike accommodation and causes the membrane potential to repolarize during the plateau phase (3). The burst terminates (4) and the repolarization phase (5) is triggered due to the convergence of several mechanisms, including accumulation of the slow AHP, the onset of glycinergic inhibition from contralateral interneurons when they are allowed to begin their cycle of activity, from small ipsilateral interneurons activated with a delay by the excitatory drive, and finally by the re-blocking of the

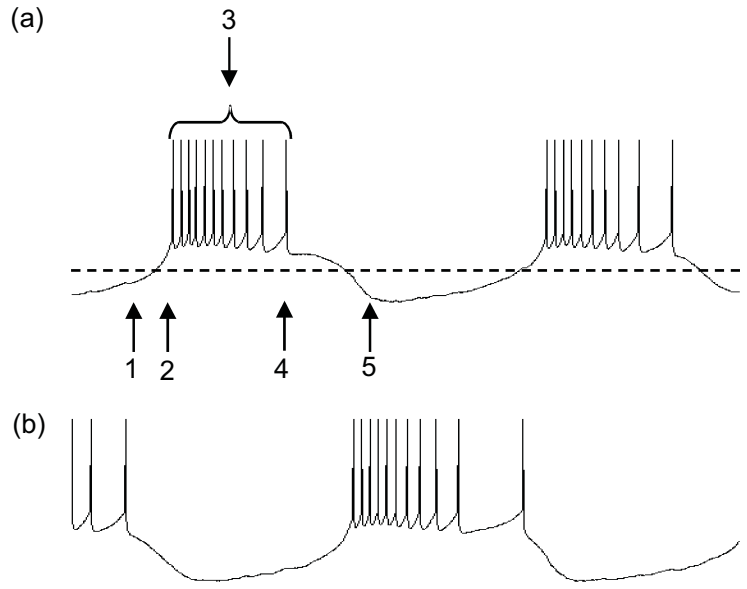

Figure 3.5 Steps in a locomotor cycle in a simulated two-cell (a, b) network coupled by reciprocal inhibition in which the two neurons are endowed with intrinsic oscillatory membrane properties. Dashed line represents background level of excitation. Simulation produced with "Neurosim" software by W.J. Heitler, University of St. Andrews, with permission. See text for description of events 1 to 5.

NMDA channels by Mg^{2+} ions. During the inter-burst period, when the membrane potential is hyperpolarized, strong conductances associated with both the trough phase of the intrinsic oscillations together with glycinergic inhibition, shunt the membrane to reduce greatly the responsiveness of motor neurons during the inter-burst period.

Outstanding Questions and Areas in Need of Further Clarification

- The spatial distribution of receptor and ion channel subtypes on the somatodendritic membrane and how this distribution affects network operation requires a deeper understanding.
- Glial cells express a variety of transporters and receptors, and are important for rapid uptake of transmitters. Little is known of their dynamic role in network function, and they may be the unsung heroes of neural network function.
- The factors involved in the differentiation of different neuron types during the development of spinal networks and how synaptic connectivity needs to be specified.

- The intrinsic role of plateau properties during fast and slow locomotor activity, and in different species, needs to be explored further.
- Although we know a lot about the effect of neuroactive substances applied exogenously, we know much less about the circumstances and extent to which these different modulatory systems are used endogenously to affect locomotion.
- Electrical coupling is prevalent in developing motor systems and declines during development, but some contribution probably remains in more mature networks. Aside from a proposed role in synchronizing activity, virtually nothing is known on the modulation of electrical coupling in the context of spinal networks function or development.
- Which building blocks can be elevated to the status of a general component of locomotor network operation must be considered.
- The way in which the spinal networks alter their architecture in response to insult or injury will be important to understand.

REFERENCES

Buchanan, J., and S. Grillner. 1988. A new class of small inhibitory interneurones in the lamprey spinal cord. *Brain Res.* **438**:404–407.

Cangiano, L., and S. Grillner. 2003. Fast and slow locomotor burst generation in the hemispinal cord of the lamprey. *J. Neurophysiol.* **89**:2931–2942.

Dale, N. 1995. Experimentally derived model for the locomotor pattern generator in the *Xenopus* embryo. *J. Physiol.* **489**:489–510.

Dale, N., and D. Gilday. 1996. Regulation of rhythmic movements by purinergic neurotransmitters in frog embryos. *Nature* **383**:259–263.

Dale, N., and F.M. Kuenzi. 1997. Ion channels and the control of swimming in the *Xenopus* embryo. *Prog. Neurobiol.* **53**:729–756.

Dale, N., O.P. Ottersen, A. Roberts, and J. Storm-Mathisen. 1986. Inhibitory neurons of a motor patter generator in *Xenopus* revealed by antibodies to glycine. *Nature* **324**: 255–257.

Dale, N., and A. Roberts. 1985. Dual component excitatory amino acid receptor-mediated synaptic potentials: Excitatory drive for swimming in *Xenopus* embryos. *J. Physiol*. **363**:35–59.

Drapeau, P., L. Saint-Amant, R.R. Buss et al. 2002. Development of the locomotor network in zebrafish. *Prog. Neurobiol.* **68**:85–111.

El Manira, A., P. Kettunen, D. Hess, and P. Krieger. 2000. Metabotropic glutamate receptors provide intrinsic modulation of the lamprey locomotor network. *Brain Res. Rev.* **40**:9–18.

Fetcho, J.R. 1991. Spinal network of the Mauthner cell. *Brain Behav. Evol.* **37**:298–316.

Grillner, S. 1985. Neurobiological bases of rhythmic motor acts in vertebrates. *Science* **228**:143–149.

Grillner, S. 2003. The motor infrastructure: From ion channels to neuronal networks. *Nat. Rev. Neurosci*. **4**:573–586.

Grillner, S., P. Wallen, R. Hill, L. Cangiano, and A. El Manira. 2001. Ion channels of importance for the locomotor pattern generation in the lamprey brainstem–spinal cord. *J. Physiol.* **533**:22–30

Hellgren, J., S. Grillner, and A. Lansner. 1992. Computer simulation of the segmental neural network generating locomotion in the lamprey by using populations of network interneurons. *Biol. Cybern*. **68**:1–13.

Hu, G.Y., Z. Biro, R.H. Hill, and S. Grillner. 2002. Intracellular QX-314 causes depression of membrane potential oscillations in lamprey spinal neurons during fictive locomotion. *J. Neurophysiol.* **87**:2676–2683.

Jonas, P., J. Bischofberger, and J. Sandkuhler. 1998. Corelease of two fast neurotransmitters at a central synapse. *Science* **281**:419–424.

Kiehn, O., J. Hounsgaard, and K.T. Sillar. 1997. Building blocks in vertebrate central pattern generators. In: Neurons, Networks, and Motor Behavior, ed. P.S.G. Stein, D. Stuart, S. Grillner, and A.I. Selverston, pp. 47–59. Boston, MA: MIT Press.

Kiehn, O., and P. Katz. 1999. Making circuits dance: Neuromodulation of motor systems. In: Beyond Neurotransmission: Neuromodulation and Its Importance for Information Processing, ed. P. Katz, pp. 275–317. Oxford: Oxford Univ. Press.

Kiehn, O., and K. Kullander. 2004. Central pattern generators deciphered by molecular genetics. *Neuron* **41**:317–321.

Kiehn, O., and M.C. Tresch. 2002. Gap junctions and motor behaviour. *TINS* **25**: 108–115.

Kozlov, A., J. Hellgren Kotaleski, E. Aurell, S. Grillner, and A. Lansner 2001. Modeling of substance P and 5-HT induced synaptic plasticity in the lamprey spinal CPG: Consequences for network pattern generation. *J. Comput. Neurosci.* **11**:183–200.

Kullander, K., S.J.B. Butt, J. Lebret et al. 2003. Role of EphA4 and EphrinB3 in local neural circuits that control walking. *Science* **299**:1889–1892.

McClellan, A.D. 2000. Neural development of motor behaviour. *Brain Res. Bull.* **Special Issue 53**:471–725.

McLean, D.L., S.M. Merrywest, and K.T. Sillar. 2000. The development of neuromodulatory systems and the maturation of motor patterns in amphibian tadpoles. *Brain Res. Bull.* **53**:595–603.

Morin, D., and D. Viala. 2002. Coordinations of locomotor and respiratory rhythms *in vitro* are critically dependent on hindlimb sensory inputs. *J. Neurosci.* **22**:4756–4765.

Orlovsky, G.N., T.G. Deliagina, and S. Grillner. 1999. Neuronal Control of Locomotion. From Mollusc to Man. Oxford: Oxford Univ. Press.

Parker, D. 2003. Activity-dependent feedforward inhibition modulates synaptic transmission in a spinal locomotor network. *J. Neurosci.* **23**:11,085–11,093.

Parker, D., and S. Grillner. 1999. Long-lasting substance-P-mediated modulation of NMDA-induced rhythmic activity in the lamprey locomotor network involves separate RNA- and protein-synthesis-dependent stages. *Eur. J. Neurosci.* **11:**1515–1522.

Parker, D., and S. Grillner. 2000. The activity-dependent plasticity of segmental and intersegmental synaptic connections in the lamprey spinal cord. *Eur. J. Neurosci.* **12**:2135–2146.

Perrins, R., and A. Roberts. 1995. Cholinergic and electrical motoneuron-to-motoneuron synapses contribute to on-cycle excitation during swimming in *Xenopus* embryos. *J. Neurophysiol.* **73**:1005–1012.

Roberts, A., S.R. Soffe, and R. Perrins. 1997. Spinal networks controlling swimming in hatchling *Xenopus* tadpoles. In: Neurons, Networks, and Motor Behavior, ed. P.S.G. Stein, D. Stuart, S. Grillner, and A.I. Selverston, pp. 82–89. Boston, MA: MIT Press.

Sillar, K.T., D.L. McLean, S.M. Merrywest, and H. Fischer. 2002. Fast inhibitory synapses: Targets for neuromodulation and development of vertebrate motor behaviour. *Brain Res. Rev.* **40**:130–140.

4

Neuromodulation of Microcircuits in Motor Systems with Special Reference to Invertebrates

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ABSTRACT

In this chapter we introduce current knowledge on neuromodulation at the various levels of analysis on which neuromodulator action becomes apparent. These span from animal behavior, to biomechanics and musculature, to neural networks, single neurons and, finally, intracellular signal cascades, genes, and protein synthesis. In contrast to neurohormones that are released into the hemolymph, neuromodulators are delivered more precisely at their target tissues. While extrinsic neuromodulation provides an independent release control, intrinsic neuromodulation can represent a more automatic release control. Neuronal networks underlying the generation of rhythmic motor patterns are known to receive a particularly rich supply of neuromodulators, and thus provide excellent case studies, some of which are presented in detail in this article.

Neuromodulators play a pivotal role in the reconfiguration of neuronal networks, and thus extend the working range of a given neuronal network enormously. Neuromodulators organize the formation of neuronal networks during development and are involved in shaping the final tuning towards their function in the adult organism. Furthermore, neuromodulators have profound effects on peripheral systems such as muscles. Not only may they change the efficacy of neuromuscular transmission, or the function of glands, but they also regulate the muscular energy metabolism, to name some examples treated here.

This chapter aims to identify some "holes" in current knowledge to guide future research. These concern in particular the following issues: (a) How are neuromodulatory neurons themselves part of larger neural networks? (b) How are these neurons activated during the respective behaviors of an organism rather than in isolated systems or *in vitro* cultures? (c) Finally, much more knowledge is required to gain insight into the particular "functional compartments" of neuromodulators released by neurons.

HISTORY AND DEFINITION OF THE TERM NEUROMODULATION

Since it was first described, the term neuromodulation has been used in various ways. Florey (1967) defined it as a "*Modulator substance* used for any compound of cellular or nonsynaptic origin that affects the excitability of nerve cells and represents a normal link in the regulatory mechanisms that govern the performance of the nervous system. Such modulator substances can affect the responsiveness of nerve cells to transsynaptic actions of presynaptic neurons and they can alter the tendency to spontaneous activity." Florey's original definition was reflected in later descriptions by Kupfermann (1979) and Kaczmarek and Levitan (1987), who stated that "neuromodulation occurs when a substance released from one neuron alters the cellular or synaptic properties of another neuron." Over time, the term took on a more general, less specific use. For example, Katz (1999) noted that "any communication between neurons, caused by release of a chemical, that is either not fast, or not point-to-point, or not simply excitation or inhibition will be classified as neuromodulatory." This statement has clear implications for the time span of a neuromodulatory action; it becomes very wide, ranging from seconds to hours, if not days and months.

By definition, neuromodulators are released from neurons (and/or glia cells); therefore, the position of a given neuromodulatory cell within the network that it modulates becomes an important issue. Katz (1999) emphasized a difference between extrinsic and intrinsic neuromodulation: for extrinsic neuromodulation, the corresponding modulatory neuron is located outside the network, whereas for intrinsic neuromodulation, the modulatory neuron is an integral part of the network itself. In fact, in the latter case, the neuromodulator may be released as a co-transmitter (Figure 4.1) and thus does not require storage in a separate class of neurons.

In some systems the neuromodulator may even be a "borrowed" transmitter which is not synthesized but taken up by the respective neuron (Musolf and Edwards, pers. comm.)

In intrinsic neuromodulation, the same information channel as in neurotransmission is used and thus may provide some kind of automatic, but time- and frequency-dependent control and release of the modulator. In addition, the co-transmitter could be involved in a feedback to the presynaptic terminal, and thus allow some processes of "self-regulation." Changes of network properties can thus be automatically induced when certain internal criteria of the network are met, for example, reaching a particular threshold or firing frequency. However, it remains a formidable task to identify these internal criteria that will trigger the release of the modulator, and hence lead to automatic changes of the network.

Extrinsic neuromodulation, by contrast, provides an additional information channel, which allows differential control and release of the modulator, and is thus independent from the activity of the network itself. Such use of a separate

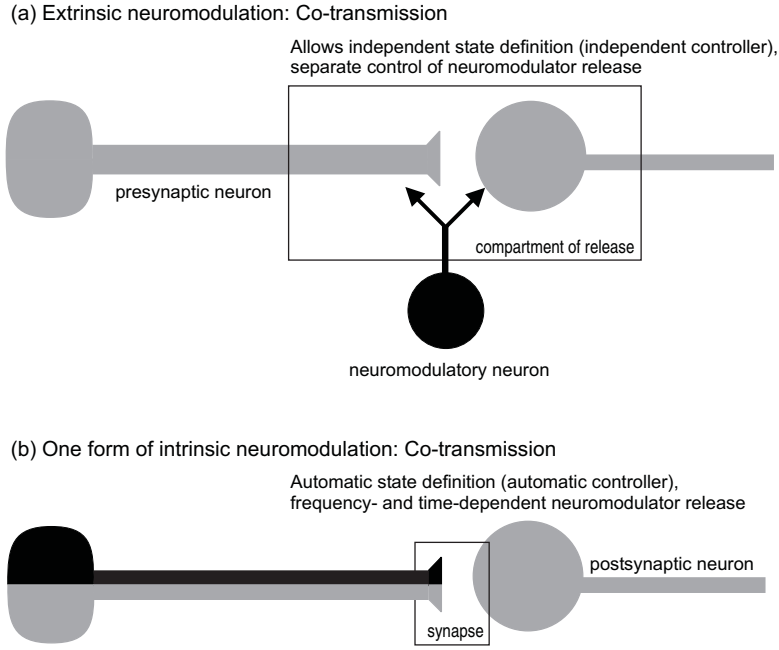

Figure 4.1 Schematic drawing of how extrinsic and intrinsic neuromodulation might provide different signaling channels to post- and presynaptic neurons. (a) Extrinsic neuromodulation: the square depicts a hypothetical compartment in which the neuromodulator acts, most likely formed by glia cells, tracheae, or other not yet understood barriers. (b) Co-transmission as an example of intrinsic neuromodulation: this compartment consists exclusively of the synaptic area.

channel may permit a more sophisticated setting and definition of different states of activity of the network. Although extrinsic neuromodulation provides a separate channel, and thus potentially an independent control of a network, many experiments show that extrinsic neuromodulatory systems are recruited in parallel to the motor systems by common presynaptic interneurons under a wide range of behavioral conditions. An important task here is to determine under which conditions the modulatory neurons couple or decouple from the activity of the network, and to identify the neurons that are responsible for this.

In recent years, the term metamodulation (or second-order neuromodulation) has been introduced to address the fact that neuromodulator release (or first-order neuromodulation) may itself be modulated (Katz 1999; Mesce 2002). Insect ecdysis is a good example where hormones, such as 20-HE (20-hydroecdysone), control the release of peptide cascades (Baker et al. 1999; Zitnan et al. 1999), which regulate the complex sequential movements required for shedding the old cuticle of the insect. Intricately intertwined cascades of peptides orchestrate the entire motor behavior, but neither all neuronal targets nor all cellular mechanisms are known. Hormones may control processes involved in

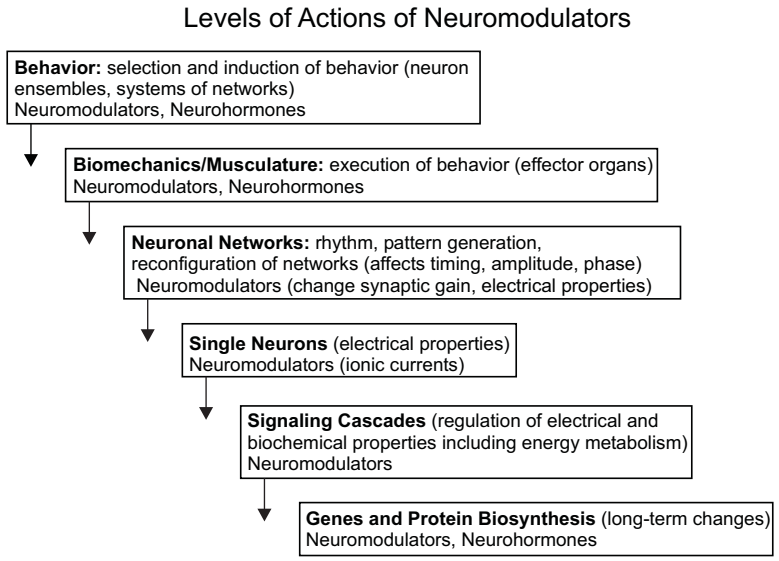
Levels of Actions of Neuromodulators
Behavior: selection and induction of behavior (neuron ensembles, systems of networks) Neuromodulators, Neurohormones
Biomechanics/Musculature: execution of behavior (effector organs) Neuromodulators, Neurohormones
Neuronal Networks: rhythm, pattern generation, reconfiguration of networks (affects timing, amplitude, phase) Neuromodulators (change synaptic gain, electrical properties)
Single Neurons (electrical properties) Neuromodulators (ionic currents)
Signaling Cascades (regulation of electrical and biochemical properties including energy metabolism) Neuromodulators
Genes and Protein Biosynthesis (long-term changes) Neuromodulators, Neurohormones

Figure 4.2 This schematic diagram depicts the different levels at which the actions of neuromodulators become obvious.

first-order neuromodulation, such as the expression or phosphorylation of neuromodulator receptors or the phosphorylation of re-uptake proteins or transporters.

Although neuromodulators always act on the cellular level, their actions or their effects have been studied on various levels of organisms (Figure 4.2). We are convinced that the behavioral importance of neuromodulator action needs to be studied on all these levels. This means that to understand the systemic (organismic) context of a neuromodulatory action is as important as to know about its cellular and molecular mechanism. In this chapter we discuss the current state of understanding neuromodulation in motor systems, with special reference to invertebrates, and identify future goals in this field.

THE ACTIONS OF NEUROMODULATORS IN MOTOR SYSTEMS

The Systems Level

Neuromodulators have been shown to affect all aspects of a neuronally generated motor program: the priming of the state of premotor networks necessary to generate a specific behavior or a particular motor output, the initiation and the maintenance of motor programs and, finally, the intensity, cycle period, and phasing of the motor output within motor programs. These influences on the systems level arise from actions of neuromodulators on sensory neurons, central

neurons, and muscle properties. For example, biogenic amines, such as octopamine and serotonin, injected into the hemolymph can determine the posture of an organism by altering the magnitude of neural output to the leg muscle control system in crayfish (Kravitz et al. 1980). Elevated levels of octopamine within the central nervous system are capable of initiating and maintaining the generation of locomotor programs, like the flight motor pattern in the locust CNS (Stevenson and Kutsch 1988). Similarly, elevated levels of octopamine can activate the leg muscle control system of the stick insect and induce active leg movements (Büschges et al. 1993). The specific effects of octopamine on motor networks led to the formation of the "orchestration hypothesis" (Sombati and Hoyle 1984; Hoyle 1985), in which a particular neuromodulator organizes more complex sequences of behavior. This is in agreement with the findings of neuromodulator effects on more complex behaviors, such as aggression (see below) or peptidergic cascades in ecdysis (see above discussion on metamodulation).

Serotonin has been shown to be of importance for the initiation and maintenance of swimming in the leech (Kristan and Nusbaum 1982), although recent experiments, where various modulators were applied to the brain of the leech, question some of those initial results (Crisp and Mesce 2003). Differential actions of various neuromodulators, such as peptides (e.g., the crustacean cardioactive peptide, CCAP, and proctolin) and biogenic amines (e.g., octopamine and 5-HT), are reported for the swimmeret system of crustaceans (Mulloney et al. 1987; Acevedo et al. 1994). Some interesting behavioral aspects of neuromodulator action come from studies on crayfish and insects where the level of neuromodulators, such as 5-HT and octopamine, may vary depending on the social status and may control the level of aggression (Kravitz and Huber 2003). One network that is particularly affected by varying 5-HT levels is the crayfish escape system, where some of the effects seen in dominant and submissive animals may be due to different expression of 5-HT receptors in component neurons of the escape network (Edwards et al. 2002). Studies on aggression in wild-type and neuromodulator-deficient *Drosophila melanogaster* will soon provide important new insights into how particular neuromodulators may be responsible for controlling whole sequences of behavior (Baier et al. 2002). As recording from the *Drosophila* CNS becomes increasingly feasible (Wilson et al. 2004), studies combining electrophysiology with genetics will undoubtedly add a new quality to understanding the behavioral role of neuromodulators.

Other interesting aspects of biogenic amines are described for honeybees in which octopamine levels in some neuropilar areas, such as the antennal lobes, are increased only in certain behavioral contexts. For example, octopamine levels increase only when foraging behavior is anticipated; they remain high during subsequent foraging, in contrast to nonforaging flights, which have no effect on octopamine levels (Schulz et al. 2002). During foraging, olfactory associative learning occurs, which itself is influenced by octopamine, most likely through the VUMmx neuron (Hammer 1993). The whole cascade of neuromodulatory

events is not yet known; however, circulating hormones (e.g., juvenile hormones) most likely play a role in orchestrating these events.

Whereas for some vertebrate motor systems it is clear that neuromodulators play a decisive role in development, maturation, and maintenance of motor patterns (see Grillner 2003; Sillar and Grillner, this volume), evidence for these types of effects in invertebrate systems is largely lacking, except for the stomatogastric ganglion (STG) of crustaceans where neuromodulators regulate the sequential expression of behavior during maturation. Neuromodulators also play most relevant roles throughout adult life in processes of plasticity: 5-HT exerts long-term structural and functional effects on neural circuits during *Aplysia* learning (Bailey et al. 2000).

The Role of Neuromodulators in Setting Cellular and Network Properties for Operation

Neuromodulators act on motor circuits by affecting the properties of the underlying neural networks and their individual neuronal elements. Alterations occur either in the excitability of neurons or the properties of their synaptic connectivity. This has been demonstrated for all consecutive layers of neuronal processing, from the level of the sensory neurons to the level of the muscle fibers generating force, thereby closing the loop for the organism–environment interaction.

The motor systems of invertebrates, ranging from mollusks to annelids and arthropods, control behaviors such as walking, flying, swimming, crawling, feeding, not to mention more complex behaviors such as prey capture or courtship. Knowledge about the underlying network and cellular mechanisms of neuromodulation is not only incomplete, but highly "patchy." This is in contrast to the potential of some of these systems, in which a large body of in-depth knowledge on the motor behaviors, the contributing component neurons, and the generation of various motor outputs has been accumulated. Component neurons of motor networks have been shown to exhibit intrinsic bursting properties upon elevated levels of neuromodulators, for example, in the presence of octopamine in the locust flight system (Ramirez and Pearson 1991a, b). The same is true for motor neurons in insect walking systems. These motor neurons can exhibit increased excitability in response to neuromodulators, like 5-HT (e.g., Parker 1995), or plateauing in the presence of elevated levels of octopamine (Ramirez and Pearson 1991a). The underlying ionic and subcellular mechanisms have not been established, but spike broadening and spike afterhyperpolarization appear to contribute to alterations in spike frequency adaptation.

One of the principle invertebrate motor circuits in which neuromodulatory action has been studied in great detail is the crustacean STG, which consists of approximately 30 neurons, primarily motor neurons of muscles moving the different parts of the stomach (Selverston et al. 1976). Several discrete rhythms generated by this network, or by subsets of its elements, have been described. Two largely distinct motor networks generate the slow rhythm of the gastric mill

(0.05–0.2 Hz) and the faster pyloric rhythm (about 1 Hz). Within the STG, most of the chemical and electrical synaptic connections as well as the electrical properties of individual neurons are well known. Different rhythms are induced because of the action of neuromodulatory neurons situated in more rostral ganglia (e.g., the commissural and the esophageal ganglia). In fact, the neuronal network within the stomatogastric network requires neuromodulatory input, as suppression of this input by blocking transmission in the afferent stomatogastric nerve blocks reversibly the gastric and pyloric rhythmic activity (Robertson and Moulins 1981).

In the pyloric network, small modifications in specific voltage-gated ionic currents of particular component neurons are important for shaping the rhythm (Harris-Warrick 2002). Neuromodulators, such as biogenic amines and neuropeptides, target these ionic currents to change the firing patterns of the network (Peck et al. 2001). Knowledge of the cellular mechanisms of peptidergic modulation is increasingly available. For example, three proctolin-containing neurons affect the pyloric rhythm differently, perhaps reflecting different co-transmitters that these neurons possess (Nusbaum et al. 2001). Thus, in the STG one has good knowledge on how particular neuromodulators or co-transmitters affect the cellular and molecular machinery of particular neurons (Harris-Warrick 2000; Nusbaum et al. 2001; Nusbaum 2002). Similarly, information on the contribution of particular ionic currents and how they are targeted by neuromodulators is presently available for some vertebrate motor systems, for example, the central pattern generators (CPGs) in the mouse spinal cord, which also has the advantage of being amenable to use the tools of genetics (Kiehn and Butt 2003).

The lack of detailed knowledge on the cellular and network mechanisms of neuromodulation in many more complex invertebrate motor circuits is in contrast to the current knowledge on specific peripheral effects of neuromodulators, for example, on the muscular system of insects and mollusks, where cellular mechanisms have been identified (see below).

Reconfiguration of Neural Networks

One functional role of neuromodulators is to act on neuronal networks in the CNS and to reconfigure them according to behavioral requirements (Harris-Warrick and Marder 1991). Thus, a certain anatomically wired network, whose component neurons are connected by either chemical or electrical synapses, can be divided upon the action of neuromodulators into functionally quite different subnetworks that serve separate behaviors or motor outputs. For example, neural networks within the CNS believed to generate rhythmic motor outputs are involved in controlling swimming, walking, crawling, stepping, and other cyclically occurring locomotor behaviors, and their action has to be modified according to the particular task. The extent to which a specific identified neuron takes part in all or only some of these behaviors remains to be determined, as demonstrated for the STG. Only the motor neurons, as output

elements, that control muscle contractions directly are activated in all these motor behaviors. This may be different for interneurons that comprise most central pattern generating as well as sensorimotor networks within the CNS. The STG as a ganglion outside the CNS may be more of an exception than the rule for central networks. The majority of interneurons are difficult to identify individually, even in invertebrates. Therefore, given the large number of interneurons, the majority are likely to belong to one of a relatively small number of groups, each with a set function. Some of them may be rather specialized and may only be involved in a special task.

Neuromodulators as Organizers during Development

Another important aspect of neuromodulators is to organize the development and maturation of the networks. This has been demonstrated for insect ecdysis (Baker et al. 1999; Zitnan et al. 1999), but recently, interesting insights have again come from studies on the development of the crustacean stomatogastric system. In the intact crustacean, the pyloric and gastric rhythms are not sequential but operate at the same time and are coupled, although their cycle frequencies are very different. In the embryo, in which the component neurons are already present and do not change in numbers, the expression of these different rhythms is suppressed due to the effects of neuromodulators. The adult rhythms associated with benthic life are progressively released only when these inhibitory influences cease during development (Casasnovas and Meyrand 1995; Fenelon et al. 1998). Therefore, in this system, neuromodulators acting on the subnetworks either tie them together, separate them from each other, or inhibit them altogether. Furthermore, neuromodulators play a decisive part in the development of the final adult patterns, and alterations in the neuromodulatory systems (e.g., production of different modulators over time by the same neuron) may alone be responsible for the observed developmental changes. Other changes may be caused by different populations of neurons being recruited by a neuromodulator, since the target neurons may only transiently express the respective receptor proteins. Information on maturation of motor patterns and the involvement of neuromodulators, in particular 5-HT, is available in vertebrates (e.g., swimming in amphibians and fish) (Sillar and Grillner, this volume). Such information is only partly available for other invertebrate motor systems. In the locust flight system, however, it has been convincingly shown that octopamine can orchestrate the CPG of flight as early as in the first larval instar when movable wings are completely absent (Stevenson and Kutsch 1988).

Peripheral Modulation, including Modulation of Synaptic and Metabolic Peripheral Targets

Important insights into how modulators act have derived not only from studies on the CNS but also on muscles, sense organs, and glands. Specific

neuromodulatory actions on the excitability of sensory neurons in response to their adequate stimuli has been shown, for example, for mechanoreceptors of insects and crayfish (Pasztor and Bush 1989; Ramirez and Orchard 1990; Ramirez et al. 1993). In an insect leg proprioceptor—the femoral chordotonal organ—octopamine was shown to increase excitability of position-sensitive sensory neurons, however, without affecting the responsiveness of velocity- sensitive sensory neurons (Ramirez et al. 1993). This suggests differential actions of neuromodulators not only on central networks but also on elements of the peripheral pathways.

Arthropod muscles are highly modulated tissues that have to produce contractions over a wide working range: from postural, tonic to fast, dynamic. Therefore, crustacean and insect muscle belong to the best-studied tissues with respect to neuromodulatory action (Rathmayer et al. 2002). Work on the peripheral role of the neuromodulator octopamine on insect muscles suggests that changing the efficacy of neuromuscular transmission is its main task. Release of octopamine during motor activity ensures that a muscle is able to perform tasks over a wider dynamic range, in particular affecting relaxation rates and, at least for many but not all insects, preventing catch effects. Thus, to alter a muscular system from postural, static, to locomotor, dynamic, control is a task of a peripheral modulator. Another task recently described by Mentel et al. (2003) is the influence of energy metabolism on the target tissue. Octopamine stimulates glycolytic rate in muscles. In flying locusts, however, flight muscles switch to lipid oxidation and use carbohydrates only at the beginning of flight. Correspondingly, the activity of dorsal unpaired median (DUM) neurons innervating flight muscles and releasing octopamine is inhibited in flying locusts. It remains to be seen how these types of neurons will function in insects that only rely on carbohydrates for their fuel during flight (e.g., flies). From this finding, it can be concluded that the action of neuromodulators may not only be exclusive to effects associated with the nervous system (e.g., synaptic transmission) but may interact with many different nonneuronal intracellular pathways. Most likely, this action is not restricted to the periphery but must surely apply to the CNS as well, as it is one of the most active metabolic tissues. To our knowledge, however, such a linkage of neuromodulators to pathways other than neuronal intracellular ones (e.g., those linked to energy metabolism) has not been addressed for the central networks.

Descending Neuromodulatory Inputs to Segmental Microcircuits

An important input to locomotory networks comes from descending neuromodulatory neurons situated in higher centers of the CNS, such as the brain or subesophageal ganglion of invertebrates or the brainstem of vertebrates. Some information exists on the function of descending inputs from 5-HT neurons of the raphe nuclei, for example, in relation to the maturation of the spinal

locomotory networks in lower vertebrates such as tadpoles (Sillar et al. 1992; Woolston et al. 1994). Pharmacological deletion of these raphe–spinal projections prevents the maturation of the normal swimming pattern (Sillar et al. 1995). In mammals, as well, 5-HT inputs seem to play an important role in spinal locomotory networks by modulating plateau properties of component neurons of spinal networks, interacting with NMDA receptors, and regulating the postspike afterhyperpolarization (Schmidt and Jordan 2000). Locomotor activity is also greatly reduced after lesions in raphe nuclei (Salles and Salles 1980). Additional evidence for the importance of inputs from raphe nuclei for locomotor patterns has resulted from the novel approach described by Ribotta et al. (2000), in which embryonic raphe cells were transplanted into the lesioned spinal cord in adult rats. This resulted in partial restoration of an otherwise severely disturbed locomotor pattern.

Extracellular single-unit recordings from raphe nuclei neurons show increased activity correlated with different and multiple motor tasks, where many units are also activated with ventilation and feeding (Veasey et al. 1995). However, the picture appears to be more complex, as shown by data from recent multi-channel, multi-neuron recordings from the dorsal raphe nucleus in awake, freely moving rats (Waterhouse et al. 2004). As far as identification of 5-HT raphe neurons is concerned, in this study these neurons exhibited low tonic discharge rates and a general insensitivity to specific sensory or motor events; non-5-HT raphe neurons, on the other hand, exhibited responses to various motor behaviors, such as locomotion, grooming, head movements, chewing, and both active and passive whisker movements. In the cat, recordings from 5-HT neurons in awake animals show a slow and regular activity (approx. 3 Hz), which may increase approximately 50% in aroused animals. Dramatic changes in the firing rates of these neurons occur during sleep (Jacobs and Fornal 1995). In addition, subpopulations of 5-HT neurons increase their activity up to fivefold when the animals chew, bite, lick, or groom, whereas the majority of 5-HT neurons do not alter their slow tonic activity. Based upon single-unit recordings in behaving animals, Jacobs and Fornal (1995) postulate that 5-HT neurons of the brain are activated exclusively in association with "gross motor activity," especially of a "tonic or repetitive nature." Clearly, more recordings, preferably from brainstem areas of lower vertebrates and from single units, are necessary to form a more complete picture of how 5-HT raphe neurons are associated with activity of spinal locomotor networks.

Another potent modulator of vertebrate locomotory systems is noradrenaline. In the *Xenopus* tadpole, noradrenaline enhances reciprocal glycinergic inhibition, which leads to a decrease in the frequency of the locomotor rhythm (Merrywest et al. 2003). A major source of the spinal noradrenergic innervation is the spinally projecting neurons located in the locus coeruleus/subcoeruleus (Proudfit and Clark 1991), which, in concert with the 5-HT raphe projections, may play an important role during development of spinal motor systems

(Tanaka et al. 1997). Recently, McLean and Sillar (2003) showed differential effects of noradrenaline on spinal locomotor networks: (a) in intact tadpoles, descending noradrenergic fibers are most likely responsible for a nonrhythmic coiling motor response with minor effects on swimming activity, whereas (b) in tadpoles where the spinal cord has been severed from the brain, noradrenaline can now release swimming behavior in response to skin stimulation. These differential actions are the result of different populations of adrenergic receptors on the respective target neurons. These results show that the activity of noradrenergic neurons may be specifically linked to various motor activities rather than exhibiting "gross motor activities."

UNSOLVED QUESTIONS AND ISSUES OF SIGNIFICANCE FOR UNDERSTANDING NEUROMODULATION IN INVERTEBRATE MOTOR SYSTEMS

The current lack of knowledge on the action of neuromodulators and the underlying signal cascades in invertebrate motor systems, with the exception of the crustacean STG and *Aplysia*, prompts investigations on all of the levels outlined in Figure 4.2. These are necessary before we can have a complete understanding of how neuromodulators act when they form and modify motor circuits, from their release and influence on the systems level to their role in modifying subcellular processes priming neuronal function. Apart from the challenging experimental and methodological approaches necessary, other issues require special attention.

In Vitro versus *in Vivo*

Many studies on neuromodulatory action are carried out on *in vitro* systems, like the STG. However, to define the "real" function of a neuromodulatory substance, we must learn how the neurons that release the respective neuromodulator are activated during an actual behavior generated. Such studies have also been performed to some extent in the STG, although data from the whole animal are rather scarce. We need to study the behavioral conditions in which a neuromodulatory neuron is active and releases its respective neuromodulator. There can be temporal, developmental, sensory (e.g., soft vs. hard food), and many other constraints. The *in vitro* findings may differ from the results of *in vivo* studies and, in particular, not all *in vitro* findings may truly reflect any behavioral relevance. A good example is the octopaminergic system in insects, where it was assumed that the neurons which release octopamine—the dorsal or ventral unpaired median neurons—are generally activated before and during motor behavior. Only when recordings from these neurons were made, during the execution of specific motor behaviors, were interesting subpopulations revealed with some neurons being inhibited rather than activated (Burrows and Pflüger

1995; Duch and Pflüger 1999). Thus, when these neurons are recorded *in vivo* in behaving animals, even under the constraints of a limited behavioral program in a more or less dissected preparation, new insights, conclusions, and ideas are likely to emerge. This particular aspect certainly has been overlooked in most of the studies of neuromodulators, and thus requires more attention in the future.

Activation of Neuromodulatory Neurons

As yet we do not know how the neuromodulatory neurons, which affect the central motor networks, are themselves activated. In the STG of crustaceans, some neuromodulatory neurons of the rostral ganglia may be activated by peripheral sense organs distributed in parts of the esophagus and stomach (Beenhakker et al. 2004). Higher brain centers may also be involved. In the case of intrinsic neuromodulators or co-transmitters, their release is tightly coupled to the motor pattern itself (see Katz 1999). One logical possibility is that modulatory neurons are activated by the command neurons or centers that activate behavior. In crustaceans and insects, some motor behavior is activated by command neurons, for example, the crayfish tail flip during escape behavior (Edwards et al. 2002) and stridulation of grasshoppers and locusts (Hedwig 2000). For stridulation, either electrical stimulation of certain brain neuropils or injection of acetylcholine can release the stridulation pattern (Heinrich et al. 2001).

The importance of the subesophageal ganglion for motor control in insects has been supported by studies on the octopaminergic system of the locust. The octopaminergic neurons are activated or inhibited in parallel with the selected motor program most likely by neurons in the subesophageal ganglion (Burrows and Pflüger 1995; Duch and Pflüger 1999). They also receive a rich synaptic drive from unknown neurons in this ganglion. In contrast to motor neurons, reflex activation of octopaminergic neurons is neither direct nor does it derive via the respective segmental ganglion but rather predominantly via the subesophageal ganglion (Field, Duch and Pflüger, in preparation). None of these activating systems has, however, been identified. This is in contrast to studies in the vertebrates, where some of the descending inputs to CPGs within the spinal cord have been identified (Sillar and Grillner, this volume).

Which Compartments of Neurons and Nervous Tissues Release Neuromodulators?

Some of the schemes developed, mainly for the STG of crustaceans located within a blood vessel, suggest that the whole network is exposed to a given neuromodulator. This may be true for this particular system, but may differ for other motor systems. Even in the STG the release may be more targeted within the neuropil. In insect and crustacean neuropils, glia cells may form effective compartments or “pools” in which neuromodulators act on all cells in possession of the respective receptor proteins but may leave other neurons outside

these borders rather unaffected (see Figure 4.1). Similarly, Nusbaum (2002) has suggested that the action of co-released biogenic amines or peptides may be affected crucially by either the respective uptake mechanisms or the presence of extracellular peptidase activity. With respect to peripheral modulation, locusts provide a good example: the octopamine levels in the hemolymph increase within the first ten minutes of flight, yet the octopamine levels of individual flight muscles decrease significantly, as do substances that normally would increase if a muscle is bathed in salines containing high octopamine concentrations (Wegener 1996). This clearly indicates that the octopamine level in the hemolymph and the octopamine level within a muscle represent two different compartments in the intact animal. Preliminary work on the ultrastructure of DUM neuron terminals (Biserova and Pflüger, unpublished) indicates that octopamine may be released towards the basal lamina in some cases and directly to the muscular membrane in others. Similar mechanisms may apply to the CNS where glial cells can provide compartmental barriers, and thus induce a flow system that directs the modulators only into a certain neuropilar area.

As stated above, neuromodulator release within the CNS is not well understood. Evidence from immunocytochemistry suggests that neuromodulators, such as octopamine, dopamine, 5-HT, histamine, and numerous peptides, are present in all major neuropils of the brain (Homberg 1994). Some neuropilar regions (e.g., those of the optical ganglia, the antennal lobes, or the central complex of the insect brain) are often densely stained by the respective antibodies, whereas others (e.g., mushroom bodies) exhibit sparse but topographically distinct staining (Sinakevitch et al. 2001). This suggests that only a subpopulation of synapses of central neurons that are part of these neuropils may be exposed to the neuromodulator, and thus may undergo modulation, whereas synapses at other locations on the same neuron may not be affected at all. Caution, however, must be applied when evidence from anatomical data is interpreted functionally. Nevertheless, there may be functional compartments for neuromodulator action within neuropils whose functional consequences are not yet understood. A similar situation may also apply to peripheral nerves. For example, in nerves innervating the STG, neuropilar structures exhibiting immunoreactivity to synaptic proteins and certain peptidergic transmitters were found (Skiebe and Ganeshina 2000); their function, however, remains to be identified.

Why So Many (Peptidergic) Neuromodulators?

In the *Aplysia* feeding circuit, nine members of the myomodulin family of peptides have been identified as co-transmitters of a motor neuron (Brezina et al. 1995). They either potentiate or depress muscle contraction; however, they may also accelerate relaxation rate. All nine myomodulins enhanced L-type Ca^{2+} currents, forming the basis of potentiation and fast relaxation of muscular contractions, whereas their effects on a specific K^+ current—the basis of depression

of muscular contractions—was much more variable. Finely tuned net effects on modulating muscle contractions were found to depend on a balance between potentiation and depression, which, most likely, reflects the different concentrations of the released myomodulins. In this system, as in many other peptidergic systems, the question arises as to why so many similar peptides exist. Several hypotheses have been proposed: different co-transmitters may be released at different locations or at different times. Reports that this may actually happen come from studies on mollusks (Benjamin and Burke 1994). Alternatively, multiple transmitters may be co-released, with each having a distinct functional contribution as each binds to a slightly different receptor that evokes a different intracellular signaling cascade. The net effects on the target tissue, therefore, would be a result of different synergistically activated receptors and intracellular pathways. Studies on the myomodulins in the *Aplysia* feeding system do not seem to support this, as all myomodulins appear to bind to one or two rather nonselective receptors. Therefore, Brezina et al. (1995) suggested that for the action on muscular contraction some members of the myomodulin family may actually be redundant, as the effects of a myomodulin mixture could more or less be mimicked by a single form of myomodulin. This redundancy may be explained by the evolutionary history of a peptide family, and as long as one form is not harmful, it may be kept rather than lost. It has been argued that unnecessary protein synthesis is energetically cheaper than its suppression would be. Retaining a redundant form may actually provide an advantage if further evolutionary development is considered, by increasing the adaptive capacity of an organism to new evolutionary constraints and processes of selection.

Another interesting aspect that has rarely been addressed is how neuromodulators released at the same time (i.e., in a "cocktail") act on the respective networks. The cocktail may have effects that cannot be explained by merely adding the effects of each component (Mesce et al. 2001; Crisp and Mesce 2003), and thus an enormous increase in "working range" of a network may occur. There is some evidence from crustacean STG that cocktails of neuromodulators are released *in vivo* (Nusbaum 2002), but this has to be more widely studied. In addition, it has to be established for all systems that the neuronal patterns studied *in vitro* correspond to those occurring *in vivo*.

SUMMARY

In this chapter, we have discussed some of the issues relevant for current knowledge, or lack thereof, with respect to understanding influences and actions of neuromodulators. We suggest that future studies should systematically address the following issues in identified systems that are eligible and appropriate for bridging the gap between subcellular mechanisms of neuromodulatory actions and their systems effects:

- The recruitment of neuromodulatory neurons and their role in initiating motor behavior, as well as the identification of the afferent and efferent connectivity of neuromodulatory neurons.
- The cellular and molecular effects of neuromodulators on target neurons of well-studied motor networks in the CNS of invertebrates.
- The definition and identification of compartments in which neuromodulators can act.
- The ontogenetic, long-term dynamics of developmental changes and effects of neuromodulatory inputs into given motor networks from larval to adult animals.

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REFERENCES

Acevedo, L.D., W.M. Hall, and B. Mulloney. 1994. Proctolin and excitation of the crayfish swimmeret system. *J. Comp. Neurol*. **345**:612–627.

Baier, A., B. Wittek, and B. Brembs. 2002. *Drosophila* as a new model organism for the neurobiology of aggression? *J. Exp. Biol*. **205**:1233–1240.

Bailey, C.H., M. Giustetto, H. Zhu, M. Chen, and E.R. Kandel. 2000. A novel function for serotonin-mediated short-term facilitation in *Aplysia*: Conversion of a transient, cell-wide homosynaptic Hebbian plasticity into a persistent, protein synthesis-independent synapse-specific enhancement. *PNAS* **97**:11,581–11,586.

Baker, J.D., S.L. McNabb, and J.W. Truman. 1999. The hormonal coordination of behavior and physiology at adult ecdysis in *Drosophila melanogaster. J. Exp. Biol.* **202**:3037–3048.

Beenhakker, M.P., D.M. Blitz, and M.P. Nusbaum. 2004. Long-lasting activation of rhythmic neuronal activity by a novel mechanosensory system in the crustacean stomatogastric nervous system. *J. Neurophysiol.* **91**:78–91.

Benjamin, P.R., and J.F. Burke. 1994. Alternative mRNA splicing of the FMRFamide gene and its role in neuropeptidergic signalling in a defined neural network. *BioEssays* **16**:335–342.

Brezina, V., B. Bank, E.C. Cropper et al. 1995. Nine members of the myomodulin family of peptide co-transmitters at the B16-ARC neuromuscular junction of *Aplysia*. *J. Neurophysiol.* **74**:54–72.

Burrows, M., and H.-J. Pflüger. 1995. Action of locust neuromodulatory neurons is coupled to specific motor patterns. *J. Neurophysiol*. **74**:347–357.

Büschges, A., R. Kittmann, and J.M. Ramirez. 1993. Octopamine effects mimick state-dependent changes in a proprioceptive feedback system. *J. Neurobiol.* **24**: 598–610.

Casasnovas, B., and P. Meyrand. 1995. Functional differentiation of adult neural circuits from a single embryonic network. *J. Neurosci.* **15**:5703–5718.

Crisp, K.M., and K.A. Mesce. 2003. To swim or not to swim: Regional effects of serotonin, octopamine and amine mixtures in the medicinal leech. *J. Comp. Physiol. A* **189**:461–470.

Duch, C., and H.-J. Pflüger. 1999. DUM neurons in locust flight: A model system for amine-mediated peripheral adjustments to the requirements of a central motor program. *J. Comp. Physiol. A* **184**:489–499.

Edwards, D.H., S.R. Yeh, B. Musolf, B.L. Antonsen, and F.B. Krasne. 2002. Metamodulation of the crayfish escape circuit. *Brain Behav. Evol*. **60**:360–369.

Fenelon, V.S., B. Casasnovas, S. Faumont, and P. Meyrand. 1998. Ontogenetic alteration in peptidergic expression within a stable neuronal population in lobster stomatogastric nervous system. *J. Comp. Neurol.* **399**:289–305.

Florey, E. 1967. Neurotransmitters and modulators in the animal kingdom. *Federation Proc.* **26**:1164–1178.

Grillner, S. 2003. The motor infrastructure: From ion channels to neuronal networks. *Nat. Rev. Neurosci*. **4**:573–586.

Hammer, M. 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* **366:** 59–63.

Harris-Warrick, R.M. 2000. Ion channels and receptors: Molecular targets for behavioral evolution. *J. Comp. Physiol. A* **186**:605–616.

Harris-Warrick, R.M. 2002. Voltage-sensitive ion channels in rhythmic motor systems. *Curr. Opin. Neurobiol.* **12**:646–651.

Harris-Warrick, R.M., and E. Marder. 1991. Modulation of neural networks for behavior. *Annu. Rev. Neurosci.* **14**:39–57.

Hedwig, B. 2000. Control of cricket stridulation by a command neuron: Efficacy depends on the behavioral state. *J. Neurophysiol.* **83**:712–722.

Heinrich, R., B. Wenzel, and N. Elsner. 2001. A role for muscarinic excitation: Control of specific singing behavior by activation of the adenylate cyclase pathway in the brain of grasshoppers. *PNAS* **98**:9919–9923.

Homberg, U. 1994. Distribution of neurotransmitters in the insect brain. In: Fortschritte der Zoologie (Progress in Zoology), ed. W. Rathmayer, vol. 40, pp. 1–88: Stuttgart: G. Fischer.

Hoyle, G. 1985. Generation of motor activity and control of behaviour: The role of the neuromodulator octopamine and the orchestration hypothesis. In: Comparative Insect Physiology, Biochemistry, and Pharmacology, ed. G.A. Kerkut and L. Gilbert, vol. 5, pp. 607–621. Toronto: Pergamon.

Jacobs, B.L., and C.A. Fornal. 1995. Activation of 5-HT neuronal activity during motor behavior. *Sem. Neurosci*. **7**:401–408

Kaczmarek, L.K., and I.B. Levitan, eds. 1987. Neuromodulation: The Biochemical Control of Neuronal Excitability. New York: Oxford Univ. Press.

Katz, P., ed. 1999. Beyond Neurotransmission: Neuromodulation and Its Importance for Information Processing. New York: Oxford Univ. Press.

Kiehn, O., and S.J.B. Butt. 2003. Physiological, anatomical, and genetic identification of CPG neurons in the developing mammalian spinal cord. *Prog. Neurobiol.* **70**: 347–361.

Kravitz, E.A., S. Glusman, R.M. Harris-Warrick et al. 1980. Amines and a peptide as neurohormones in lobsters: Actions on neuromuscular preparations and preliminary behavioural studies. *J. Exp. Biol.* **89**:159–175.

Kravitz, E.A., and R. Huber. 2003. Aggression in invertebrates. *Curr. Opin. Neurobiol*. **13**:736–743.

Kristan, W.B., Jr, and M.P. Nusbaum. 1982. The dual role of serotonin in leech swimming. *J. Physiol.* (*Paris*) **78**:743–747.

Kupfermann, I. 1979. Modulatory actions of neurotransmitters. *Annu. Rev. Neurosci.* **2**:447–465.

McLean, D.L., and K.T. Sillar. 2003. Spinal and supraspinal functions of noradrenaline in the frog embryo: Consequences for motor behaviour. *J. Physiol.* **551**.2:575–587.

Mentel, T., C. Duch, H. Stypa et al. 2003. Central modulatory neurons control fuel selection in flight muscle of migratory locust. *J. Neurosci*. **23**:1109–1113.

Merrywest, S.D., J.R. McDearmid, O. Kjaerulff, O. Kiehn and K.T. Sillar. 2003. Mechanisms underlying the noradrenergic modulation of longitudinal coordination during swimming in *Xenopus laevis* tadpoles. *Eur. J. Neurosci*. **17**:1013–1022.

Mesce, K. 2002. Metamodulation of the biogenic amines: Second-order modulation by steroid hormones and amine cocktails. *Brain Behav. Evol*. **60**:339–349.

Mesce, K.A., K.M. Crisp, and L.S. Gilchrist. 2001. Mixtures of octopamine and serotonin have nonadditive effects on the CNS of the medicinal leech. *J. Neurophysiol.* **85**:2039–2046.

Mulloney, B., L.D. Acevedo, and A.G. Bradbury. 1987. Modulation of the crayfish swimmeret rhythm by octopamine and the neuropeptide proctolin. *J. Neurophysiol.* **58**:584–597.

Nusbaum, M.P. 2002. Regulating peptidergic modulation of rhythmically active circuits. *Brain Behav. Evol.* **60**:378–387.

Nusbaum, M.P., D.M. Bitz, A.M. Swensen, D. Wood, and E. Marder. 2001. The roles of co-transmission in neural network modulation. *TINS* **24**:146–154.

Parker, D. 1995. Serotonergic modulation of locust motor neurons. *J. Neurophysiol.* **73**:923–932.

Pasztor, V.M., and B.M. Bush. 1989. Primary afferent responses of a crustacean mechanoreceptor are modulated by proctolin, octopamine, and serotonin. *J. Neurobiol*. **20**:234–254.

Peck, J.H., S.T. Nakanishi, R. Yaple, and R.M. Harris-Warrick. 2001. Amine modulation of the transient potassium current in identified cells of the lobster stomatogastric ganglion. *J. Neurophysiol*. **86**:2957–2965.

Proudfit, H.K., and F.M. Clark. 1991. The projections of locus coeruleus neurons to the spinal cord. *Prog. Brain Res.* **88**:123–141.

Ramirez, J.M., A. Büschges, and R. Kittmann. 1993. Octopaminergic modulation of the femoral chordotonal organ in the stick insect. *J. Comp. Physiol. A* **173**:209–219.

Ramirez, J.M., and I. Orchard. 1990. Octopaminergic modulation of the forewing stretch receptor in the locust Locusta migratoria. *J. Exp. Biol*. **149**:255–279.

Ramirez, J.M., and K.G. Pearson. 1991a. Octopamine induces bursting and plateau potentials in insect neurones. *Brain Res.* **549**:332–337.

Ramirez, J.M., and K.G. Pearson. 1991b. Octopaminergic modulation of interneurons in the flight system of the locust. *J. Neurophysiol.* **66**:1522–1537.

Rathmayer, W., S. Djokaj, A. Gaydukov, and S. Kreissl. 2002. The neuromuscular junctions of the slow and the fast excitatory axon in the closer of the crab *Eriphia spinifrons* are endowed with different Ca^{2+} channel types and allow neuron-specific modulation of transmitter release by two neuropeptides. *J. Neurosci.* **22**:708–717.

Ribotta, M.G., J. Provencher, D. Feraboli-Lohnherr et al. 2000. Activation of locomotion in adult chronic spinal rats is achieved by transplantation of embryonic of embryonic raphe cells reinnervating a precise lumbar level. *J. Neurosci.* **20**:5144–5152.

Robertson, R.M., and M. Moulins. 1981. Oscillatory command input to the motor pattern generators of the crustacean stomatogastric ganglion. I. The pyloric rhythm. *J. Comp. Physiol. A* **143**:453–463.

Salles, M.S., and K.S. Salles. 1980. Behavioral effect of selective and non-selective lesions of median raphe nucleus in the rat. *Jpn. J. Physiol.* **30**:105–114.

Schmidt, B.J., and L.M. Jordan. 2000. The role of serotonin in reflex modulation and locomotor rhythm production in the mammalian spinal cord. *Brain Res. Bull*. **53**: 689–710.

Schulz, D.J., A.B. Barron, and G.E. Robinson. 2002. A role for octopamine in honey bee division of labor. *Brain Behav. Evol.* **60**:350–359.

Selverston, A.I., D.F. Russell, and J.P. Miller. 1976. The stomatogastric nervous system: Structure and function of a small neural network. *Prog. Neurobiol.* **7**:215–290.

Sillar, K.T., J.F. Wedderburn, and A.J. Simmers. 1992. Modulation of swimming rhythmicity by 5-hydroxytryptamin during post-embryonic development in *Xenopus laevis*. *Proc. R. Soc. Lond. B* **250**:107–114.

Sillar, K.T., A.M. Woolston, and J.F. Wedderburn. 1995. Involvement of brainstem serotonergic interneurons in the development of a vertebrate spinal locomotor circuit. *Proc. R. Soc. Lond. B* **259**:65–70.

Sinakevitch, I., S.M. Farris, and N.J. Strausfeld. 2001. Taurine-, aspartate, and glutamate-like immunoreactivity identifies chemically distinct subdivisions of Kenyon cells in the cockroach mushroom body. *J. Comp. Neurol.* **439**:352–367.

Skiebe, P., and O. Ganeshina. 2000. Synaptic neuropil in nerves of the crustacean stomatogastric nervous: An immunocytochemical and electron microscopic study. *J. Comp. Neurol.* **420**:373–397.

Sombati, S., and G. Hoyle. 1984. Generation of specific behaviors in the locust by local release into neuropil of the natural neuromodulator octopamine. *J. Neurobiol.* **15**: 481–506.

Stevenson, P.A., and W. Kutsch. 1988. Demonstration of functional connectivity of the flight motor system in all stages of the locust. *J. Comp. Physiol. A* **162**: 247–259.

Tanaka, H., S. Takahashi, and J. Oki. 1997. Developmental regulation of spinal motoneurons by monoaminergic nerve fibers. *J. Periph. Nerv. Syst.* **2**:323–332.

Veasey, S.C., C.A. Fornal, C.W. Metzler, and B.L. Jacobs. 1995. Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *J. Neurosci.* **15**:5346–5359.

Waterhouse, B.D., D. Devilbiss, S. Seiple, and R. Markowitz. 2004. Sensorimotor-related discharge of simultaneously recorded, single neurons in the dorsal raphe nucleus of the awake, unrestrained rat. *Brain Res.* **1000(1–2)**:183–191.

Wegener, G. 1996. Flying insects: Model systems in exercise physiology. *Experientia* **52**:404–412.

Wilson, R.I., G.C. Turner, and G. Laurent. 2004. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science* **303**:366–370.

Woolston, A.M., J.F. Wedderburn, and K.T. Sillar. 1994. Descending serotonergic spinal projections and modulation of locomotor rhythmicity in *Rana temporaria* embryos. *Proc. R. Soc. Lond. B* **255**:73–79.

Zitnan, D., L.S. Ross, I. Zitnanova et al. 1999. Steroid induction of a peptide hormone gene leads to orchestration of a defined behavioral sequence. *Neuron* **23**:523–535.

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5

Group Report: Microcircuits in the Motor System

O. KIEHN, Rapporteur

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INTRODUCTION

In our discussion of the microcircuits in motor systems, we focused on rhythmic movements and saccadic eye movements with an emphasis on vertebrate systems. Behaviors studied by the members of the group include *swimming* (lamprey, tadpole), *walking* (rodent, stick insect), *respiration* (cat, rodent), *flight* (locust), and *saccadic eye movements* (rodent, cat, monkey). After initial discussions about how to define a microcircuit, we decided not to be restrictive in our definition but rather to use a relatively broad definition, defining microcircuits as networks that generate specific motor acts. We addressed the following major themes:

- Network structure in sample motor systems.
- Architecture at the cellular and network level: What are the essential building blocks and how do they contribute to the behavior?
- What can we learn from studying the assembly of central pattern generating (CPG) neurons during development?
- Modulation at cellular and network level.

NETWORK STRUCTURE IN SAMPLE MOTOR SYSTEMS

To gain insight into the general organization of motor circuits in motor systems, we considered network structure in examples of motor systems that operate in different modes: CPG networks which produce slow rhythmic outputs (e.g., swimming, walking, and respiration) and a motor system which produces fast motor acts, namely, saccadic eye movements. These networks are presented below in formalized diagrams. A short description introduces each network.

Neither the diagram nor the description is meant to capture all elements of the network function, but rather to highlight key features of the network organization. For example, the description ignores, for the time being, all cellular properties. It should be stressed that in some networks, certain connections have only been inferred from the overall network output, neuronal firing, and cross correlation rather than being demonstrated directly by paired recordings.

Swimming CPGs (Tadpole, Lamprey)

Swimming is an undulatory rhythmic movement that requires activation of muscles in alternation on either side of the body. The cycle frequency of swimming ranges from 0.2–10 Hz in the lamprey and from 10–20 Hz in the tadpole. The basic structure of the swimming CPG network is illustrated by Figures 5.1 and 5.2, which depict the CPG networks in the tadpole and lamprey (Grillner 2003; Roberts et al. 1998). The similarities between the two CPGs are striking. In the lamprey, it has been shown that each one of the spinal segments contains a CPG unit, with CPG neurons on both the left and right side of the cord. The overall structure of the CPG unit is composed of two types of interneurons: excitatory glutamatergic neurons and inhibitory glycinergic commissural interneurons. Excitatory glutamatergic neurons provide monosynaptic excitation of motor neurons. Commissural interneurons inhibit contralateral neurons by axons crossing in the midline to ensure segmental alternation: when one side is active, all neurons on the other side are silenced. Finally, CPG activity is initiated by descending fibers originating in reticulospinal neurons in the brain. For further details, see Sillar and Grillner (this volume).

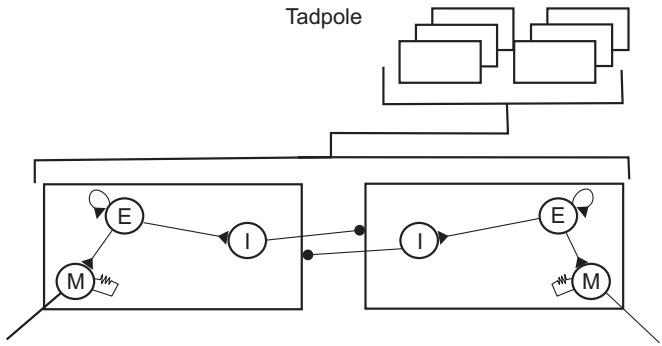

Figure 5.1 Basic schematic representation of the microcircuits in the spinal locomotor networks of the tadpole. Note that on each side of the spinal cord, as shown on the right top side, an excitatory kernel is located that interacts with the contralateral side via mutual inhibition (for details, see Sillar and Grillner, this volume). The details of the central pattern generating network are shown at the bottom. E: excitatory interneuron; I: inhibitory interneuron; M: motor neuron; circles denote groups of neuron types; ▲ denote excitatory synapses; ● denote inhibitory synapses; zigzag lines denote coupling via electrical synapses.

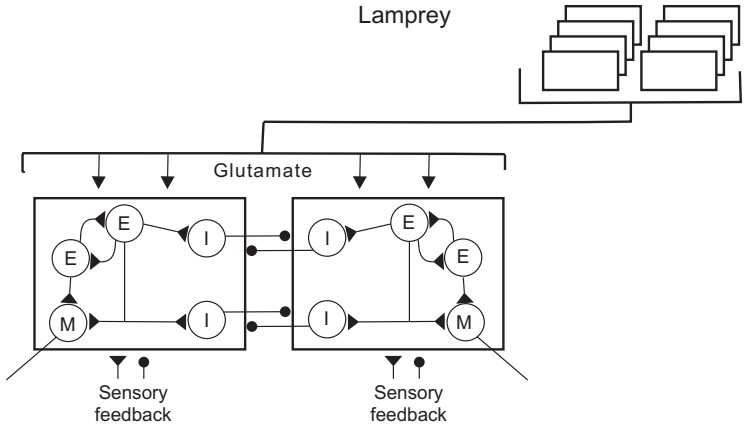

Figure 5.2 Basic schematic representation of the microcircuits in the spinal locomotor network of the lamprey. Within each hemi-segment of the spinal cord, an excitatory kernel is located that interacts with the contralateral hemi-segment via mutual inhibition, as shown on the right top side (for details, see Sillar and Grillner, this volume). Detailed topology of the segmental CPG is shown at the bottom. E: excitatory interneuron; I: inhibitory interneuron; M: motor neuron; circles denote groups of neuron types; ▲ denote excitatory synapses; ● denote inhibitory synapses; arrows denote influences.

Walking CPGs

The coordination of walking is different from that of swimming because, in addition to the requirement for left–right coordination, there is also a need for flexor–extensor alternation and proximal–distal coordination in the limb. Locomotor frequencies are relatively slow, in the range of 0.2–3 Hz, depending on the animal (Bässler 1983; Graham 1985; Orlovsky et al. 1999). This general organization of a walking CPG can be illustrated in the stick insect (Figure 5.3). Here, the leg muscle control system for walking is located in the thoracic nervous system, with each leg being controlled by an individual neural controller with a modular structure (for a review, see Bässler and Büschges 1998). The main modules are constituted by neural networks governing the individual leg joints, namely the thorax-coxa joint, the coxa-trochanter, and the femur-tibia joint. Within each module, a CPG network that is able to generate alternating activity in the antagonistic motor neuron (E and F) pools supplying a leg joint represents a kernel (Bässler et al. 2003; for a review, see Bässler and Büschges 1998). Some of the interneurons in the CPG have been identified (Büschges 1995). As in many CPGs (e.g., Akay et al. 2001; Hess and Büschges 1999), sensory signals from movement and strain sensors on each locomotor organ take part in organizing the overall motor output by intra- and interjoint influences on the timing of the individual joint CPGs, as well as by determining the intensity of motor activity (e.g., Bucher et al. 2003). Current knowledge indicates that during network

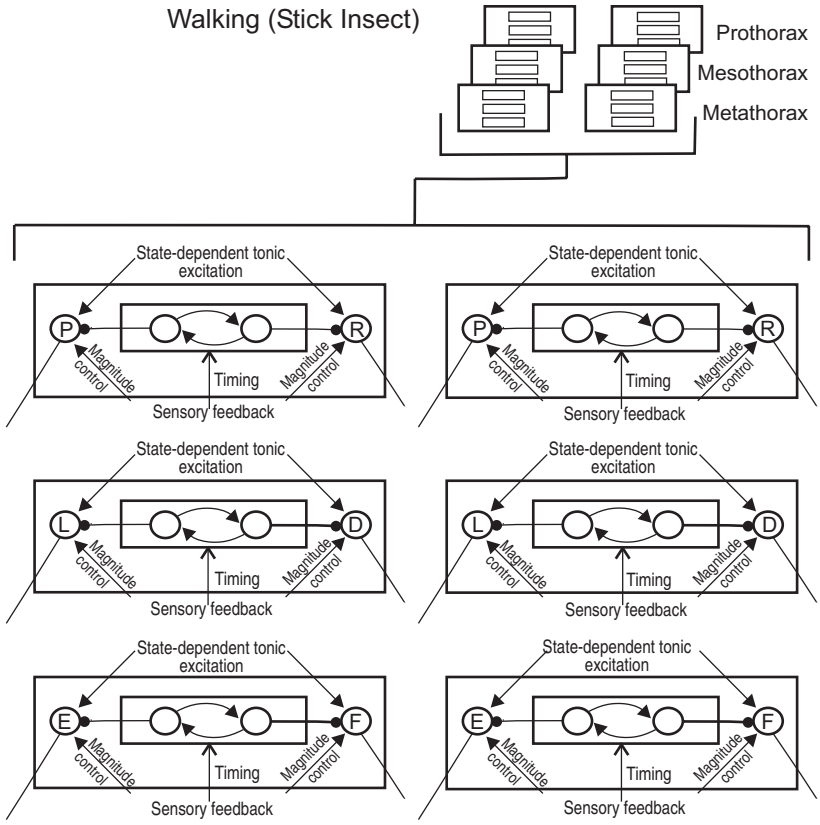

Figure 5.3 Schematic representation of the microcircuits in the thoracic locomotor network of the stick insect. Note that within each hemi-ganglion of the pro-, meso-, and meta-thoracic ganglion, at least three microcircuits are located, as shown on the right top side (for details, see text). The circuitry of the three hemi-segmental microcircuits, each governing one of the three main leg joints, is shown at the bottom. There is no prominent influence between contralateral microcircuits. P: protractor; R: retractor; L: levator; D: depressor; E: extensor tibiae motor neuron; F: flexor tibiae motor neuron; circles denote groups of neuron types; empty circles indicate CPG neurons in the module; • denote inhibitory synapses; arrows illustrate influences.

operation, rhythmic activity in leg motor neurons results from a general tonic excitation together with alternating phasic excitatory and inhibitory synaptic inputs, the latter being provided by the joint CPGs (Büschges 1998; Büschges et al. 2004). Repolarization of individual motor neurons from hyperpolarization is assisted by an intrinsic depolarizing sag potential (Schmidt et al. 2001). Descending commands from the brain are responsible for initiation and maintenance of walking, as well as basic modification of the segmental motor program for adaptive modifications, like optomotor-induced turns.

Respiratory CPG

In mammals, the respiratory rhythm underlying breathing is a coordinated alternation of inspiratory, postinspiratory, and expiratory phase activities (for a review, see Richter and Spyer 2001). The rhythm is slow (ca. 0.1–2 Hz) and overlaps the dynamic range of other rhythmic motor systems. The CPG consists of a *hybrid pacemaker network* (for recent reviews, see Smith 1997; Smith et al. 2000) in which endogenous pre- or early-inspiratory (pre-/early-I) burster cells represent the excitatory kernel that activates the populations of early-inspiratory (early-I) and ramp-inspiratory (ramp-I) neurons (Figure 5.4). In addition, the network receives tonic excitatory drive from extrinsic sources. The burster cells are embedded in the network and go through inhibitory synaptic control. Other neurons of the network, such as early-I and postinspiratory (PI) neurons, also show a tendency to endogenous bursting. Maintenance and augmentation of respiratory phase activities, such as early-I and ramp inspiratory (ramp-I), postinspiratory (PI), and expiratory (E) activities, are guaranteed by recurrent excitatory connections. Many synaptic connections between neurons are, however, inhibitory and reveal reciprocal or recurrent circuitries (see recurrent activation

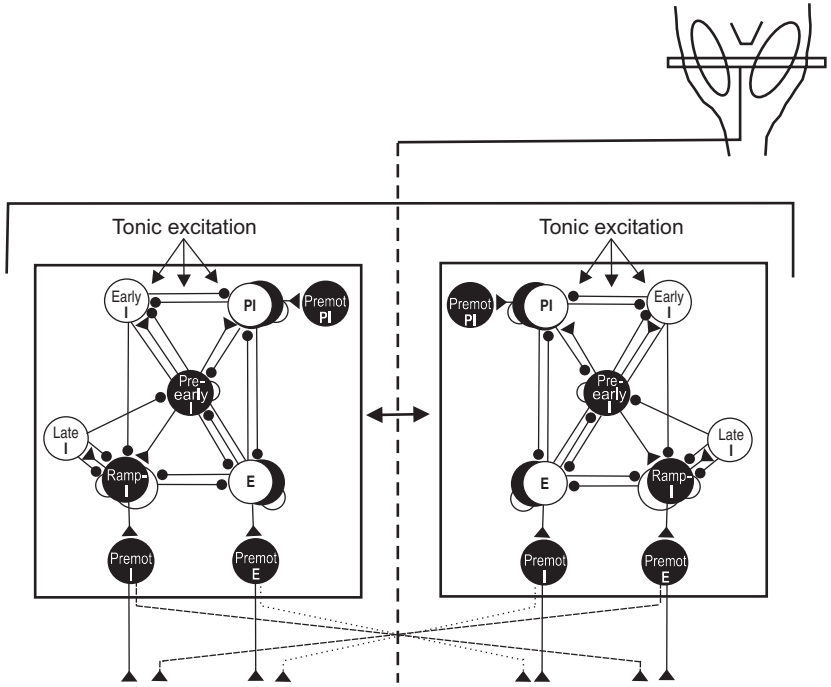

Figure 5.4 Schematic representation of the microcircuits in the mammalian brainstem respiratory network (see text for details). The detailed topology of the bilaterally located CPG network is shown at the bottom. PI: postinspiratory neurons; E: expiratory activities; early-I: early-inspiratory neurons; ramp-I: ramp-inspiratory neurons.

of inhibitory late-inspiratory neurons). Ramp-I, PI, and E neurons also have their excitatory counterparts, which provide excitatory drive to spinal and cranial motor neurons. The microcircuits are located bilaterally within the pre-Bötzinger complex and adjacent regions of the ventral group of respiratory neurons. Both half-centers receive tonic excitatory drive from external sources and are strongly synchronized through commissural excitatory interconnections. Bulbospinal premotor neurons send ipsi- and contralateral projections to spinal motor neurons innervating respiratory muscles or to ipsilateral cranial motor neurons innervating glossopharyngeal, hypoglossal, or laryngeal muscles. The connections of a large variety of afferent inputs (e.g., from the lungs and chemoreceptors) and the feedback control mechanisms (e.g., pontine respiratory regions and dorsal group of respiratory neurons) (Richter and Spyer 2001) are not considered in the schematic.

Saccadic Eye Movements (Mammals)

Saccades are rapid eye movements used for capturing the image of a visual target in the space of the fovea. Peak velocities of saccades reach several hundred degrees per sec. The CPGs of saccadic eye movements are located in the superior colliculus (SC) and the brainstem reticular formation (Scudder et al. 2002; Sparks and Hartwich-Young 1989; Sparks et al. 2000) (Figure 5.5). Here we focus on the microcircuits of the SC. Initiation of a saccade is triggered by a phasic burst in fast-spiking neurons of the deeper layer of the SC (E neurons). The bursts are generated by a recurrent excitatory network, in which NMDA receptor-mediated synaptic transmission and release from tonic GABAergic inhibition by the substantia nigra pars reticulata (I SNr) and local interneurons are

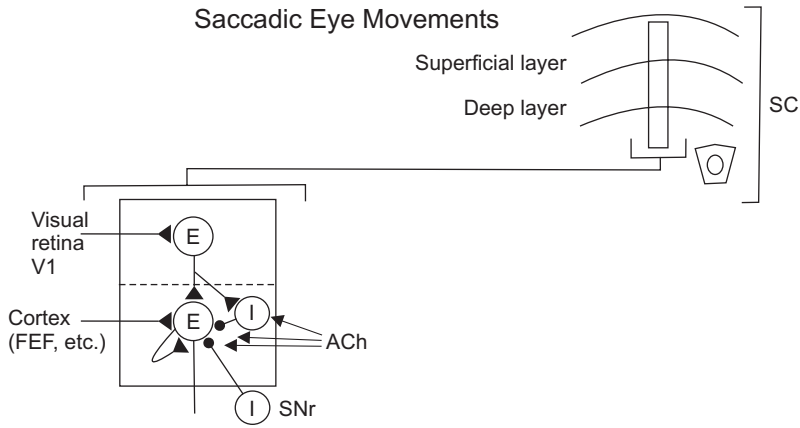

Figure 5.5 Schematic representation of the microcircuits in the mammalian superior colliculus (SC). ACh: acetylcholine; E: excitatory; FEF: frontal eye field; I: inhibitory; SNr: substantia nigra pars reticulata.

critical (Saito and Isa 2003). In addition, cholinergic inputs lower the threshold for burst generation (Aizawa et al. 1999). Each burst neuron discharges before a large range of eye movements and, accordingly, a large population of neurons is active before and during each saccade (Sparks 1978). Disruptions of the spatial and temporal profile of population activity modify, in a predictable manner, the probability of saccade occurrence as well as the direction and amplitude of the movement (Lee et al. 1988). This indicates that each neuron in the active population contributes to the metrics of the movement, and these data are consistent with a vector-averaging scheme for extracting information from the collicular command (intracollicular contribution; see Isa and Sparks, this volume). In the SC the maximum amplitude and direction of a saccade vector are determined primarily by location of the active cell population on the map (Robinson 1972). The place-coded output signal from the SC is transformed into the dynamics of saccades by the brainstem saccade generator circuit, where transient release from tonic glycinergic inhibition by omnipause neurons triggers high-frequency bursts in excitatory and inhibitory burst neurons, which drive and suppress extraocular motor neurons, respectively (Keller 1979; Scudder et al. 2002; Sparks 2002).

ARCHITECTURE AT THE CELLULAR AND NETWORK LEVEL: WHAT ARE THE ESSENTIAL BUILDING BLOCKS AND HOW DO THEY CONTRIBUTE TO THE BEHAVIOR?

Network Functional Architecture

Networks generating rhythmic motor behavior seem to have a functional architecture that is designed to generate—through dynamic interactions of intrinsic cellular and synaptic mechanisms—two fundamental aspects of the motor behavior: (a) *a network oscillation (rhythm*) exhibited by all of the network neurons, and (b) *a spatiotemporal pattern* of activity of (pre)motor neurons that is appropriate for coordinated activation of the muscles producing the movements. For example, locomotor networks generate a rhythmic cycle of activity/motor output that is temporally coordinated, so that there is alternation between activity on opposite sides of the spinal cord; in vertebrates with limbs, there is also alternation between motor output activating functionally antagonistic muscle groups. Similarly, in the mammalian respiratory network, the cycle consists of three alternating phases: inspiration, postinspiratory, and expiration, with a detailed spatiotemporal pattern of activity (see previous section). Thus it appears that the network can be described as having a global network oscillation that provides the temporal framework of the rhythmic cycle, and a network component that is structured to generate the spatiotemporal coordination of (pre)motor neuron activity throughout the cycle. Accordingly, current experimental and

modeling approaches should be designed to address questions about network architecture regarding rhythm generation and pattern formation in at least three main areas:

1. *Localization of neuron populations involved in rhythm and pattern generation.* Where in the network are the major elements generating the rhythm and spatiotemporal pattern, and what is the connection architecture between these elements?
2. *Mechanisms of rhythm generation*. How are the network components generating the rhythm distributed and functionally organized? What are the critical cellular and synaptic mechanisms? The debate centers on questions of whether rhythm generation involves: (a) network(s) of excitatory neurons with intrinsic bursting pacemaker properties; (b) a network of inhibitory interneurons with rhythm emerging from network synaptic interactions; and (c) a combination of the above, where the mechanisms operating can be dependent on the network's state (e.g., experimentally reduced network vs. intact network).
3. *Spatiotemporal pattern formation.* What are the cellular and synaptic mechanisms involved in shaping and coordinating the temporal patterns of neuron activity throughout the rhythmic cycle of network activity?

All of the networks considered earlier (see NETWORK STRUCTURE IN SAMPLE MOTOR SYSTEMS) consist of interacting assemblies of excitatory and inhibitory interneurons organized as functional modules. Therefore, a major question that emerged from our discussions was: Is there a common functional organization regarding excitatory and inhibitory network mechanisms?

Excitatory Interneuron Populations: A Mechanistic Core, or Excitatory "Kernel," for Rhythm Generation and Excitatory Drive Formation

Excitatory drive has been shown to contribute to the activation and generation of rhythmic and episodic activity in many microcircuits in the motor systems in vertebrate and invertebrate model systems. A tonic component of the excitatory drive has been described to contribute, for example, in the networks of the leech swimming (Marder and Calabrese 1996), the stomatogastric nervous system (Marder and Calabrese 1996), the locust flight system (Hedwig and Pearson 1984), the stick insect walking system (Büschges 1998; Büschges et al. 2004), the lamprey and the tadpole spinal locomotor systems (see Sillar and Grillner, this volume), the mammalian respiratory system (Richter and Spyer 2001, Smith 1997, 2000), the SC (Isa and Sparks, this volume), and the mammalian spinal cord (see references in Kiehn and Butt 2003; Kiehn et al. 1997). In addition, phasic excitation plays a prominent role in rhythm generation and excitatory drive formation. Because of the available in-depth knowledge on the mammalian respiratory network and the vertebrate spinal locomotor networks, phasic excitation was discussed in greater detail by the group.

The central concept that emerged is that a population of interconnected excitatory interneurons functions as a kernel that generates rhythmic excitatory (glutamatergic) synaptic drive, in the case of lamprey and tadpole (and possibly also the mammalian locomotor CPG), directly to motor neurons and, in the case of the respiratory network to motor neurons, through an additional layer of premotor neurons (Figure 5.4). These excitatory interneurons are clustered and interconnected unilaterally in each spinal cord segment (locomotor networks) or, in the case of the respiratory network, in circumscribed bilateral regions of the brainstem, called the pre-Bötzinger complex. As proof of this concept, in four systems—lamprey locomotor network (Cangiano and Grillner 2002), tadpole locomotor network (Roberts et al. 1998), rat locomotor network (Kudo and Yamada 1987), and respiratory network (Smith et al. 1991, 2000)—it has been demonstrated experimentally that this kernel can be isolated physically (hemisegment of spinal cord *in vitro*, slice isolating the pre-Bötzinger complex *in vitro*) and that the kernel can generate a stable network rhythm in the absence of inhibitory synaptic mechanisms. Thus the kernel in these systems has either intrinsic autorhythmic or pacemaker-like properties, or other sets of voltage-dependent membrane conductance that impose rhythmogenic properties (see section below on CELLULAR FUNCTIONAL ARCHITECTURE). In the case of the respiratory network, this inherent voltage-dependent rhythm-bursting mechanism has been show to represent a potentially important mechanism for frequency control at the cellular level, which in turn underlies frequency control at the population level (e.g., when the population is driven by tonic excitation) (Koshiya and Smith 1999). Although there is limited experimental evidence, modeling studies (Butera et al. 1999a, b) suggest that rhythmic bursting is synchronized within the kernel by excitatory glutamatergic synaptic connections. Presumably, the various cellular membrane properties that promote oscillatory bursting also promote synchronization of bursting within the excitatory network. Thus, in general, rhythm generation in this kernel emerges from dynamic interactions of cellular properties and fast excitatory synaptic connections, providing the most rudimentary mechanism for rhythmic burst generation. The kernel, by definition, represents a mechanistic core on which control mechanisms can be added and overlaid. Thus these excitatory cells are proposed to possess not only intrinsic cellular and synaptic mechanisms that promote rhythm generation, but to function as substrates for rhythm control by a wide variety of superimposed cellular/synaptic input mechanisms, including synaptic inputs from inhibitory interneuron populations, which in the intact network regulate bursting behavior of the kernel.

Inhibitory Interneurons: Functional Elements for Pattern Formation

In all of the systems considered, it appears that the excitatory kernel is embedded in a network of inhibitory interneurons. That is, there is a functional overlay and

temporal interaction of synaptic inhibition with the network elements generating and transmitting excitatory synaptic drive. The excitatory kernel drives some (or all) of the inhibitory neurons. Although there is little direct experimental evidence for this theoretically, recurrent connections between inhibitory interneurons could serve to synchronize their activity. At the level of the excitatory kernel, this phasic inhibition from glycinergic/GABAergic connections becomes fundamentally involved in rhythm generation by controlling the membrane potential trajectory and resetting rhythmic bursting of the kernel neurons to phase transitions in the network. In general, these inhibitory interneurons are required for temporally shaping the discharge patterns of (pre)motor neurons during a given phase of network activity, and are critically required to produce the coordinated phase transitions that give rise to alternating patterns of activity between different phases of network activity. For example, in locomotor networks, bilaterally distributed, reciprocally connected populations of commissural inhibitory interneurons produce alternating activity states between the two sides of the spinal cord. Ipsilateral projecting interneurons probably serve additional coordinating roles in the walking CPG. In the respiratory network, reciprocal inhibitory synaptic interactions allow the transition between and generation of the inspiratory, postinspiratory, and expiratory phases of network activity enabling the orderly temporal evolution of the characteristic multiphase pattern of network activity during the respiratory cycle (Richter and Spyer 2001). Thus, there is a consensus that the inhibitory interconnections function in both rhythm control and neuronal discharge pattern formation. Although not investigated at length, we propose that the inhibitory network neurons express voltage-dependent conductances that contribute importantly to discharge formation in the inhibitory network, promote postinhibitory rebound bursting required for stable phase transitions between activity of reciprocally connected populations of inhibitory neurons, and facilitate burst generation in response to the kernel's phasic excitatory synaptic drive (see below).

Modular Architecture of Rhythm and Pattern Generation (Micro)circuits: Building Blocks of Larger-scale Networks underlying Spatially Distributed Rhythmic Motor Behavior

The assembly of interacting excitatory and inhibitory neurons, indicated by the block diagrams (Figures 5.1–5.5), are proposed to represent functional modules. How exactly these are organized (e.g., in the mammalian walking CPG) is not known. However, in the case of the swimming CPG, it appears that each module of excitatory kernel and associated complement of inhibitory interneurons is duplicated on each side of a given spinal segment, and that these modules are distributed longitudinally along the spinal cord, allowing spatiotemporal coordination of rhythm and pattern generation of all longitudinally distributed motor neurons engaged in locomotor behavior. In the case of the

respiratory network, each modular network is distributed bilaterally but not arrayed spatially along the neuraxis (see Sillar and Grillner, this volume). A modular arrangement is also observed in the stick insect. In principle, each module can function autonomously to generate a basic pattern of rhythmic network activity by virtue of the excitatory kernel; however, coupling of modules is required to generate the coordinated, bilaterally distributed patterns underlying the complete spatiotemporal pattern of rhythmic motor activity responsible for behavior.

Network Architecture of Saccade Generator Circuits

The general architecture of the saccadic system differs from that of CPGs involved in rhythmical locomotor movements. A place-coded signal generated in the SC is transformed into separate, rate-coded signals that control the amplitude of the horizontal (pons) and vertical (rostral midbrain) components of the saccade (Scudder et al. 2002; Sparks 2002). Unlike rhythmical movements, in which one component of the movement ends when the next component begins, the termination of a saccade is controlled by a classical feedback circuit that uses a copy of the motor command as the feedback signal. The excitatory drive to burst neurons in the pons and rostral midbrain is reduced to zero when the corollary discharge signal of the "actual" eye displacement matches the signal of the desired eye displacement. The cellular and network components of the feedback control of saccade amplitude are poorly understood.

The interactions between the visual signals observed in the superficial layers and the motor commands generated by cells in deeper layers has been controversial (Edwards 1980; Isa 2002; Mays and Sparks 1980). That such interactions occur is now well established, and these interactions may be particularly important during movements with short reaction times (Aizawa et al. 1999; Isa et al. 1998; Lee et al. 1997). Saccadic reaction times exhibit bimodal distributions. Reaction times of "regular saccades" range between 150 and 250 ms, while on particular occasions (e.g., when the target is presented a few hundred milliseconds after the subject is released from fixation), saccades with extremely short latencies (80–120 ms) called "express saccades" are observed (Fischer and Boch 1983). It has been supposed that the visual responses of superficial layers (latency: 40–50 ms) fail to induce motor burst activities in the deeper-layer neurons (Dorris et al. 1997; Sparks et al. 2000). In contrast, in the case of express saccades, it is likely that the deeper-layer neurons are sufficiently disinhibited beforehand and, as a consequence, visual responses of the superficial layer neurons trigger motor bursts in the deeper layer. It has also been suggested that the cholinergic input facilitates such gating of the signal transmission through the interlaminar pathway (Aizawa et al. 1999). Two clear examples of (anatomical) modularity in this system are the regularly spaced clumps of nigral input to the intermediate collicular layers (Graybiel 1978; Illing and Graybiel 1985), also

visible in cholinergic enzyme stains (Graybiel 1978b; Ma et al. 1981). These patterns suggest that input functions related to nigral and cholinergic inputs are also modular.

Network Analysis in Complex Motor Networks

There was general agreement in our group that a major issue in a complex network is to define neurons and their connectivity. This is a manageable task in small invertebrate motor networks with few neurons, like the stomatogastric ganglion (STG), which contains less than 30 neurons. However, in larger motor networks, the more indirect way to determine whether a neuron is a member of the network is to record extra- or intracellularly from cells and relate the cells firing to the actual motor act. Additionally, the synaptic projection pattern can be determined by dual recordings or correlation analysis and the network might be deduced from this in combination with modeling studies. In vertebrates, this approach has been successful in the locomotor systems of lamprey and tadpoles and in respiratory CPG. When it comes to the mammalian walking CPG, however, there is lack of understanding about the precise network structure. Early studies have identified Ia and Renshaw cells as rhythmically active, possibly playing a role in shaping the ipsilateral motor output (Pratt and Jordan 1987). More recent studies have also revealed the network connectivity of the commissural interneurons in the rodent (Butt et al. 2002a, b; Butt and Kiehn 2003; Eide et al. 1999; Kiehn and Butt 2003; Stokke et al. 2002) and the cat (Bannatyne et al. 2003; Jankowska et al. 2003). The commissural interneurons in the mammalian spinal cord is a mixed population with both segmental and intersegmental connections of both excitatory and inhibitory connections onto motor neurons. The commissural interneurons seem to be driven by the excitatory kernel and are thus involved in pattern generation. At the moment, few excitatory CPG neurons have been identified in the mammalian CPG (Hultborn et al. 1998; Kiehn and Butt 2003). Further advances of these studies will be greatly facilitated when specific molecular markers become available for neuron subclasses (see section below: WHAT CAN WE LEARN FROM STUDYING THE ASSEMBLY OF MOTOR MICROCIRCUITS DURING DEVELOPMENT?). Then cells can be recognized after intracellular dye fills or in the mouse being labeled with GFP, CFP, etc., to visualize them directly in slices or intact preparations (see Kiehn and Butt 2003; Kiehn and Kullander 2004).

Cellular Functional Architecture

One of the important problems in the analysis of microcircuits is to define the palette of conductances in functionally defined neurons and in the different parts of their dendritic arbor. The discussion of these issues is hampered by the fact that we only have incomplete knowledge about the palette of conductance, even in the best-studied systems. Because of time constraints, we focused mainly on

the adult lamprey and mammalian respiratory systems, where it has been possible not only to describe the ionic conductances in considerable detail, but also to develop biophysically realistic models of neurons' electrophysiological "behavior." Equivalent knowledge also exists for the *Xenopus* embryo swimming CPG (Dale and Kuenzi 1997). This system provides an interesting comparison, particularly with the lamprey, where the equivalent channels play subtly different roles. For example, K_{Ca} channels in the lamprey are important for spike frequency regulation and the control of NMDA oscillations (see below); in the tadpole, however, these channels have much slower kinetics and contribute to the duration of the whole episode of swimming. For comparison with the two slow rhythmic systems, we also considered some of the ionic mechanisms described in the SC. Examples of cellular properties in these three systems, as far as they are currently understood, are discussed below.

The Locomotor System of the Lamprey

Neurons in the locomotor system of lamprey fire in bursts and at relatively low rates (around 10–70 Hz). They have a significant postspike afterhyperpolarization (imAHP) due to K_{Ca} (SK3) channels, which summates during repetitive activity and serves as the major factor underlying frequency modulation in these neurons and thereby frequency adaptation (Sillar and Grillner, this volume). The smaller the imAHP, the smaller the adaptation will become. In addition to the K_{Ca} component, there is also a sodium-dependent K^+ component (K_{Na}) with the same time course: it is very small after single spikes but becomes much larger after a burst when it may represent 50% of the imAHP. These two channels are important for determining the duration of activity of CPG neurons. Lamprey neurons do not have the very slow AHP (sAHP) in hippocampal neurons, and the imAHP seems to be the important player in the adult spinal microcircuits. In lamprey neurons, K_{Ca} channels are activated through N and P/Q type Ca^{2+} channels, both of which are the targets of modulation via several transmitters, including 5-HT, dopamine, and $GABA_B$. They all indirectly reduce the imAHP, and thereby frequency adaptation, and contribute to burst prolongation.

The action potential duration in lamprey neurons is under tight control both in the soma and in the axon terminal through activation of several K^+ channel subtypes (delayed rectifier, K_V 3.4, fast K_{Na}) (El Manira et al. 2002; Kettunen et al. 2003). These channels are subject to modulation. This is important to maintain reproducibility with regard to Ca^{2+} entry during the action potential, which controls both the transmitter exocytosis at the synapse and the amplitude of the imAHP.

Voltage-dependent properties of NMDA channels allow for plateau properties during burst activity and boost synaptic inputs (Wallen and Grillner 1987). The plateaus are terminated by activation of K_{Ca} through the NMDA-induced Ca^{2+} entry. In the lamprey network, K_{Ca} channels thus play a crucial role for

burst termination, both in the intact network and in the excitatory kernel (for references, see Sillar and Grillner, this volume).

A synaptic input occurring far out in the dendritic tree would be markedly attenuated at the spike-initiating site in the initial segment if the dendritic tree was purely passive. However, several active voltage-dependent mechanisms have been shown to counteract this effect, such as dendritic Na^+ channels (in lamprey by 20%; Hu et al. 2002), low voltage-activated Ca^{2+} channels, and also NMDA channels. In other systems, boosting of dendritic potentials is mediated by a mixture of a low-threshold L-like calcium current and a persistent sodium current (Heckman et al. 2003; Kiehn and Eken 1998). Any modulation of K^+ channels will also markedly affect the degree of attenuation by affecting the overall membrane impedance and thereby the length constant. Dendritic processing and configuration are also important for neuronal integration. The density of different ion channel subtypes along the dendrites will profoundly affect neuronal function and synaptic integration. At present, the data for these different systems is limited mainly to information based on recordings from intact neurons, compared to isolated dissociated neurons with no or a very limited dendritic tree. In common with many other systems, both invertebrate and vertebrate, synaptic inhibition (glycine or GABA) in lamprey appears to be located at the soma or proximal dendrites, whereas glutamatergic excitation tends to be at a more distal location. K_{Ca} channels appear also to be located primarily on proximal dendrites rather than soma.

The Respiratory System

The respiratory network consists of a group of excitatory glutamatergic neurons in the pre-Bötzinger complex embedded in a tripartite network of inhibitory neurons. The excitatory neurons have voltage-dependent pacemaker properties and mutually excite each other. An important current underlying these oscillations is a persistent sodium channel that rapidly activates and slowly inactivates (Del Negro et al. 2002). The inspiratory phase is initiated in part by activation of the current followed by a progressive inactivation. Computer models (Butera et al. 1999a, b) of the biophysical mechanisms underlying the oscillatory bursting of these cells suggest that this conductance is never fully inactivated over the timescale of the respiratory cycle, and the dynamics of the recovery from inactivation importantly regulates the duration of the interburst period when the kernel is isolated from inhibitory synaptic control. The frequency of oscillation is also affected by the degree of background excitatory drive and inhibitory synaptic control and is regulated importantly by G-protein-coupled potassium leak conductances, such as Kir and TASK, which are subject to neuromodulation. These cells have high voltage-activated calcium currents (Mironov and Richter 1998; Pierrefiche et al. 1999) and K_{Ca} channels, which may contribute to burst termination (Busselberg et al. 2003). Other calcium-activated currents, such as

CAN currents, may also contribute to rhythmic burst generation, and it remains an important problem to determine the roles of calcium fluxes and sets of currents that are dependent on these fluxes in the excitatory kernel neurons.

Other neurons of the network revealing bursting behavior show reactivation of a transient calcium current following release from chloride-mediated synaptic inhibition and membrane depolarization, which leads to activation of P-type calcium current (Ramirez and Richter 1996). Action potential discharge during the bursts activates L-type calcium currents which lead to intracellular Ca^{2+} accumulation reaching average concentrations of 200–300 nM (Frermann et al. 1999). This activates SK and BK Ca currents, which contributes to spike frequency adaptation of PI neurons (Pierrefiche et al. 1995) and phase termination of inspiration (Busselberg et al. 2003). The latter mechanism contributes to an efficient inhibitory synaptic mechanism of phase switching.

The Saccade System in the Superior Colliculus

In the case of the saccade generator, ionic conductances playing a key role in burst generation are not fully understood. The output neurons in the motor layer are designed to fire at high frequency (up to 1000 Hz), have brief action potentials, and a small afterhyperpolarization. It is clear that NMDA receptor-mediated synaptic transmission is essential for burst generation of deeper-layer neurons in the SC (Saito and Isa 2003). On the other hand, Ca^{2+}-dependent plateau potentials may not be essential because bursts can still be observed when the intrapipette solution contains BAPTA. Low threshold Ca^{2+} channels are observed only in a minority of deeper-layer neurons; however, the contribution of the low threshold Ca^{2+} channels in triggering the initiation of bursts in a subpopulation of the deep layer neurons is not excluded. A hyperpolarization-activated inward current (Ih) is expressed heavily in a subpopulation of SC neurons, namely, the wide field vertical cells. HCN channels in this subclass of neurons are densely expressed in their wide dendritic trees and continuously depolarize their dendrites to near-threshold level for dendritic spike generation, rather than contributing to rhythm generation by assisting the rebound depolarization. Less is known about the cellular mechanisms involved in the termination of saccades (Endo et al. 2003). Whether the cessation of the saccade-related burst of neurons in the SC is produced by inhibitory feedback input is unknown.

WHAT CAN WE LEARN FROM STUDYING THE ASSEMBLY OF MOTOR MICROCIRCUITS DURING DEVELOPMENT?

During ontogeny, neuronal microcircuits that contribute to the production of motor behavior need to be assembled. The study of how microcircuits emerge during development is advantageous in the quest to understand the cellular and

network basis of adult motor behavior for several reasons. First, the networks are constructed very early in development. The network building blocks on which adult networks are based might be added in layers, and these building blocks can therefore be studied more readily. Second, these early embryonic networks gradually acquire the complexity required to produce sophisticated adult motor behavior and their sequential modification (e.g., through the addition of modulatory inputs or through changes in the complement of receptors and ion channels) can inform us about why adult networks are built in particular ways. Third, by making careful interspecies comparisons of how the maturation process unfolds, it becomes possible to identify not only the critical branch points at which species-specific aspects of circuit design and function diverge, but also features that are common and which might therefore represent general principles.

Some general principles were identified from our analysis:

1. Assembly of rhythm-generating modules occurs very early in development, even before the actual behavior they control. This has been shown in almost all species studied (for references, see Nishimaru and Kudo 2000; Sillar et al. 1997).
2. The excitatory kernel develops earlier than the inhibitory network, most clearly demonstrated from experiments in the rodent (Hilaire and Duron 1999; Nishimaru et al. 1996).
3. The timing of descending modulatory inputs is coincident with important motor events and might play an instrumental role in modulating the motor output (McLean et al. 2000).
4. Motor output flexibility and complexity increases during development (McLean et al. 2000).
5. Neurons often change their transmitter phenotype during development.

As a result, the effect of a given neuron (class) can alter dramatically. A well-known example is the developmental switch from GABA to glycine found in many vertebrate microcircuits (Nishimaru and Kudo 2000). This switch indicates a change in the operational mode of the network because $GABA_A$-operated channels often provide depolarization early in development. This suggests that activation of $GABA_B$ receptors might play a more active role in coordination early in development (Zhang et al. 1999).

Complicating the task of identifying any general principles that might exist is the presence of a set of specific cellular and synaptic changes, which may or may not be species specific. For example, while descending monoaminergic fibers in the tadpole spinal cord contribute to the development of the spinal CPG from an immature to a more mature state (McLean et al. 2000), neuromodulatory substances in the crustacean STG seem to suppress the complexity of the CPG network output in the larva so that it expresses an immature pattern from an adult STG network already laid down early in development (Fenelon et al. 2003).

More knowledge is clearly needed to understand how the final compliment of receptors and ion channels present in adult networks are selected during development from the large palette potentially available. Moreover, the developmental signals responsible for orchestrating this timetable of changes are almost wholly unknown. One interesting possibility is that there are causal links between neuromodulatory inputs to developing networks and the trophic changes that establish their detailed wiring. For example, there is evidence that 5-HT is causally linked to the dynamic changes that occur during network maturation and also to the establishment of synaptic connectivity upon which network output depends. An important area for future study will be to assimilate a detailed understanding of the temporal acquisition of multiple transmitters and the functional consequences of their release upon motor networks. In addition to changes in the transmitter phenotype of neurons embedded in motor networks, there are complex and as yet poorly understood temporal and spatial changes in the receptors and in channels upon which neurotransmitters act. These changes can be broadly categorized into (a) changes in absolute and relative receptor densities; (b) addition of new ion channels and receptors; and (c) changes in the subunit composition of existing receptors/ion channels that alter function in significant ways (e.g., NMDA subunits). Studies of such changes are an area for future research in the context of motor microcircuits.

Another area of developmental studies is the use of genetic tools to identify subpopulations of network neurons. Several groups (Goulding et al. 2002; Jessell 2000; Lee and Pfaff 2001; Sharma and Peng 2001) have shown that a large fraction of the ventrally located interneurons (INs) in the rodent spinal cord are derived from four early classes of ventral embryonic neurons, designated V0-V3 INs. V1 and V2 INs are ipsilaterally projecting while V0 and V3 INs mainly project commissurally. The four groups of interneurons are characterized by a unique set of transcription factors early in development: V0s express Evx1, V1 expresses En1, V2s express either Gata3 or Chx10, and V3s express Sim1. The promoters of such transcription factors can be used selectively to modify or kill groups of neurons. There are three methods that can be used: cell knockout or genetic ablation, electrical silencing of cells, and specific block of synaptic release (for references, see Kiehn and Butt 2003). *Genetic ablation* is obtained with expression of the diphtheria toxin A subunit (DTA), which interferes with protein synthesis. *Electrical silencing* is based on overexpressing the cloned genes of natural or modified K^+ (or Cl^-) channels of several different types in neurons. Introducing the channel causes a hyperpolarizing shunt so that neuronal activity is suppressed during network activity. *Blocking synaptic release* is another way of functionally deleting neurons from a network. This can be done by targeted expression of tetanus toxin light chain (TeTxLC), which blocks action potential-evoked neurotransmitter release.

Specific promoters can also be used to drive the expression of neuronal reporters, like GFP and CFP, which can be used to identify populations of cells. By expressing several different reporters (especially those that also label axons), it

might be possible with time lapse to study the assembly of the microcircuits in cultures of early embryonic tissue.

Definitions pose a major problem. Currently, all the known transcription factors define heterogeneous groups of cells, encompassing more than one assembly of interneurons. For example, deleting a heterogeneous population of neurons is obviously not going to tell us much about their function in the network. Thus, for the genetic manipulation approach to be successful, we need to define neuronal units through a combination of genetic, anatomical, and electrophysiological (functional) properties (Kiehn and Kullander 2004). By using the regulatory sequences of shared genes in these units they can be inactivated either during development or transiently in adult mice. Since the network activity is directly coded in behavior, the motor networks are ideal places to test such genetic strategies. An obvious limitation to these kinds of techniques is that thus far they can only be used in a small number of species (mouse, zebrafish, *Drosophila melanogaster*).

MODULATION AT CELLULAR AND NETWORK LEVEL

The output of any neural network cannot be fully explained by the intrinsic electrical properties and synaptic connections alone. To account for the full working range of a network, it is necessary to incorporate adaptive mechanisms to accommodate changing behavioral requirements. The group recognized and discussed the significance of neuromodulation in this process. In addition, we agreed that neuromodulatory input is important for the development, the initiation, and the maintenance of motor patterns (Casasnovas and Meyrand 1995). However, it became clear that there is no single unifying definition of what neuromodulation actually is. Nevertheless, it is necessary to implement a working definition and, for the purposes of this discussion, we define neuromodulation as *the targeted release of a substance from a neuron (or glial cell) that either alters the efficacy of synaptic transmission or the cellular properties of a pre- and/or postsynaptic neuron (or glia) via metabotropic receptors*.

An enormous amount of information has already accrued regarding specific details of how various neuromodulators act at the cellular level within motor networks and about how the same neuromodulators affect motor output when applied exogenously (Harris-Warrick and Marder 1991; Katz and Harris-Warrick 1999). However, with the exception of certain motor systems, particularly in invertebrates (see Pflüger and Büschges, this volume), there is incomplete knowledge about how and when neuromodulatory neurons are activated, where they act within the microcircuits, and how different neuromodulators exert effects in combination to determine the final motor output (cf. below).

One of the more complete pictures of neuromodulatory action is provided by the locust in which octopamine initiates a particular motor behavior, namely

flight (Sombati and Hoyle 1984). In addition, in this system recording from neuromodulatory neurons during restrained or fictive motor behavior reveals several important aspects of the organizational link of these neurons to the motor microcircuits:

- Neuromodulatory neurons are activated in parallel with the motor network, and the CPG provides synaptic input for neuromodulatory neurons as these remain active even during fictive locomotion (Baudoux et al. 1998; Burrows and Pflüger 1995).
- The strong coupling of neuromodulatory neurons to motor patterns ensures a phasic activation, which, most likely, is particularly efficient in releasing high amounts of modulators.
- The recordings from these neurons during ongoing motor behavior not only reveal their role in altering the efficacy of synaptic transmission but also in affecting pathways of energy metabolism. Thus, intracellular recordings from these neuromodulatory neurons during motor behavior yielded some important insights into their functional role (Duch et al. 1999; Mentel et al. 2003).

Similar studies of neuromodulatory neurons in other systems (e.g., the lamprey or the rat) may also be rewarding, since in these more complex systems it will be more difficult to ascertain principles of neuromodulatory action. To begin to consider the levels of action of neuromodulation, it might be useful to reduce these vertebrate microcircuits to the two major components already discussed: the excitatory "kernel," which generates rhythmicity, and the overlying inhibitory networks, which coordinate the motor pattern. In principle, there are two conceptually different (though mutually interacting) ways in which neuromodulators can alter the properties of a network: regulating synaptic strengths or regulating the ion conductance that determines the integrative properties of postsynaptic neurons. It is possible that a general principle of neuromodulation in motor networks is that the inhibitory components are subject to synaptic modulation, whereas the excitatory kernel and its downstream motor pools are modulated predominantly by postsynaptic mechanisms.

The output of virtually all motor networks spans a range of possible output configurations in which the frequency and intensity can be graded between two extremes of performance. In one case these different extremes may be accomplished by two different amines (noradrenaline (NA), 5-HT), which exert opposing effects on swimming activity in frog larvae. Apparently, the opposing effects of these amines on frequency are accomplished via presynaptic modulation of the strength of reciprocal glycinergic inhibitory connections (McLean et al. 2000). Serotonin weakens synaptic transmission to reduce cycle periods, whereas NA strengthens the same connections to lengthen cycle periods (McDearmid et al. 1997). In addition to these effects, which target the inhibitory component, the two amines also influence the excitatory kernel through

postsynaptic mechanisms that are complementary to the final motor output they produce. However, in this system, as for other vertebrate systems in which this type of modulation has been studied, the mechanism through which endogenously released NA and 5-HT exert this form of control has not been determined in detail. Work on the lamprey has shown that serotonergic ventral plexus neurons are active during fictive swimming and that the 5-HT they release exerts a profound influence on the motor output by blocking K_{Ca} channels (Sillar and Grillner, this volume). There are also extensive studies of unit recordings from both raphe and locus coeruleus neurons in the cat, which demonstrate that they are activated during motor activity (Jacobs and Fornal 1997). Taken together, these studies mainly show a correlation with the motor behavior, without investigating the precise site of action in the network. Moreover, the higher-order control of the modulatory systems is poorly understood. It may only be possible to answer these questions by recording from identified neuromodulatory neurons in well-defined behavior states while simultaneously monitoring motor output. Such studies are starting to be performed in the SC. The only modulatory system that has been studied in the deeper layer of the SC is the cholinergic input, originating from the pedunculopontine tegmental nucleus (PPTN). At the network level, the cholinergic inputs are supposed to facilitate the burst generation; however, the cellular level of analysis is still fragmental. Three different types of activities were observed in the PPTN during performance of visually guided saccade task: (a) activities related to preparation and execution of saccades, (b) tonic activities related to motivation level of the monkeys, and (c) activities related to reward delivery (Kobayashi et al. 2002). A more recent study suggested that neurons with the second activity pattern had broad spike width, suggesting that they are cholinergic neurons. Other transmitter systems, such as enkephalin and substance P, have been shown to innervate a deeper layer of the SC (Graybiel et al. 1984; Illing 1992); however, their physiological role has not been clarified.

Serotonergic Modulation of the Respiratory CPG

The respiratory network receives intensive innervation of serotonergic afferents and is modulated persistently by elevated 5-HT levels in the VRG region. Persistent elevation of 5-HT levels correlates with the tonic discharge of raphe neurons, which, among others, receive excitatory inputs from respiratory neurons (Richter et al. 1999). Individual respiratory neurons are very sensitive to 5-HT, because they express various receptor isoforms of the 5-HT_{1A}, $5\text{-HT}_{2a,\ b}$, 5-HT_{4a}, and 5-HT_{7} subtype. 5-HT_{1A} receptors are most extensively expressed and dominate. This explains why the genuine transmitter 5-HT or its analogs depress effectively the excitability of respiratory neurons through activation of K conductances and augmentation of inhibitory synaptic currents. Any disturbances of inhibitory synaptic processes leading to failure of inspiratory off-

switching and prolongation of inspiratory bursting (apneusis) can, therefore, be treated by activation of 5-HT_{1A} receptors (Richter et al. 2003). Interestingly, there is evidence that inhibitory early-I neurons are specifically sensitive to 5-HT_{1A} receptor modulation. This has important consequences for the network. When early-I neurons are suppressed by 5-HT, inhibitory PI neurons respond with a shift of their periodic discharge into the inspiratory phase. As a result, there is depression of the postinspiratory phase and the respiratory rhythm oscillates only between two phases: inspiration and expiration.

The Gs-coupled 5-HT_4 and 5-HT_7 receptors counteract the Gi-mediated depression of adenylate cyclases by 5-HT_{1A} receptors (and also the effect of many others, e.g., μ opioid receptors). Such elevation of cytosolic cAMP levels enhances the excitability of respiratory neurons and even overcomes fentanyl-induced depression of breathing and apnea (Manzke et al. 2003). 5-HT_2 receptors are also widely expressed in the respiratory network. Activation of these receptors or their intracellular PLCβ-DAG–IP3 signal pathway increases neuronal excitability, as seen in membrane depolarization and augmentation of excitatory synaptic currents (Lalley et al. 1995). 5-HT seems to play a specific role during ontogeny as there are significant variations in expression patterns of specific receptor isoforms during embryonic development. Part of these trophic effects seems to be mediated by 5-HT_7R–G12 and 5-HT_4R–G13 signaling pathways targeting small GTPases of the Rho family, RhoA and Cdc42, and regulating neuronal outgrowth and retraction (Ponimaskin et al. 2002).

CONCLUSIONS

It has been possible to elucidate the microcircuits of motor systems to an arguably unprecedented level of detail, when compared to the other microcircuits surveyed in this volume. These motor microcircuits have the advantages of being confined to relatively accessible parts of the nervous system and of being responsible for specific, identifiable forms of behavior. Moreover, despite the fact that the preparations used to study motor circuitry are very different and the behaviors they control diverse, it has nevertheless been possible to compare and contrast them, and hence to identify common principles of design and operation. In virtually all systems discussed, at both immature and adult stages, we identified the existence of an excitatory kernel of (glutamatergic) interneurons responsible for generating phasic bursting in motor neurons that are involved in both rhythmic and episodic behaviors. Burst generation relies on the presence of specific sets of ionic conductances, some of which are common and some specific to particular motor systems. This bursting activity does not equate to real behavior, however, and a more behaviorally relevant motor pattern is sculpted by the superimposition of inhibitory (glycinergic) interneurons, which play the key role of coordinating the activity of different components of the

microcircuits. The excitatory kernel and inhibitory pattern generator appears to develop separately and might also be subject to differential neuromodulation.

REFERENCES

Aizawa, H., Y. Kobayashi, M. Yamamoto, and T. Isa. 1999. Injection of nicotine into the superior colliculus facilitates occurrence of express saccades in monkeys. *J. Neurophysiol*. **82**:1642–1646.

Akay, T., U. Bässler, P. Gerharz, and A. Büschges. 2001. The role of sensory signals from the insect coxa-trochanteral joint in controlling motor activity of the femur-tibia joint. *J. Neurophysiol*. **85**:594–604.

Bannatyne, B.A., S.A. Edgley, I. Hammar, E. Jankowska, and D.J. Maxwell. 2003. Networks of inhibitory and excitatory commissural interneurons mediating crossed reticulospinal actions. *Eur. J. Neurosci*. **18**:2273–2284.

Bässler, U. 1983. Neuronal Basis of Elementary Behavior in Stick Insects. Berlin: Springer.

Bässler, U., and A. Büschges. 1998. Pattern generation for stick insect walking movements: Multisensory control of a locomotor program. *Brain Res. Rev.* **27**:65–88.

Bässler, U., A.E. Sauer, and A. Büschges. 2003. Vibration signals from the FT joint can induce phase transitions in both directions in motoneuron pools of the stick insect walking system. *J. Neurobiol*. **56**:125–138.

Baudoux, S., C. Duch, and O.T. Morris. 1998. Coupling of efferent neuromodulatory neurons to rhythmical leg motor activity in the locust. *J. Neurophysiol*. **79**:361–370.

Bucher, D., T. Akay, R.A. DiCaprio, and A. Büschges. 2003. Interjoint coordination in the stick insect leg-control system: The role of positional signaling. *J. Neurophysiol*. **89**:1245–1255.

Burrows, M., and H.-J. Pflüger. 1995. Action of locust neuromodulatory neurons is coupled to specific motor patterns. *J. Neurophysiol*. **74**:347–357.

Büschges, A. 1995. Role of local nonspiking interneurons in the generation of rhythmic motor activity in the stick insect. *J. Neurobiol*. **27**:488–512.

Büschges, A. 1998. Inhibitory synaptic drive patterns motoneuronal activity in rhythmic preparations of isolated thoracic ganglia in the stick insect. *Brain Res*. **783**:262–271.

Büschges, A., B. Ludwar, D. Bucher, J. Schmidt, and R.A. DiCaprio. 2004. Synaptic drive contributing to rhythmic activation of motoneurons in the deafferented stick insect walking system. *Eur. J. Neurosci.* **19**:1856–1862.

Busselberg, D., A.M. Bischoff, and D.W. Richter. 2003. A combined blockade of glycine and calcium-dependent potassium channels abolishes the respiratory rhythm. *Neuroscience* **122**:831–841.

Butera, R.J., Jr., J. Rinzel, and J.C. Smith. 1999a. Models of respiratory rhythm generation in the pre-Bötzinger complex. I. Bursting pacemaker neurons. *J. Neurophysiol.* **82**:382–397.

Butera, R.J., Jr., J. Rinzel, and J.C. Smith. 1999b. Models of respiratory rhythm generation in the pre-Bötzinger complex. II. Populations of coupled pacemaker neurons. *J. Neurophysiol.* **82**:398–415.

Butt, S.J., R.M. Harris-Warrick, and O. Kiehn. 2002a. Firing properties of identified interneuron populations in the mammalian hindlimb central pattern generator. *J. Neurosci*. **22**:9961–9971.

Butt, S.J., and O. Kiehn. 2003. Functional identification of interneurons responsible for left-right coordination of hindlimbs in mammals. *Neuron* **386**:953–963.

Butt, S.J., J. Lebret, and O. Kiehn. 2002b. Organization of left-right coordination in the mammalian locomotor network. *Brain Res. Rev.* **40:**107–117.

Cangiano, L., and S. Grillner. 2002. Fast and slow locomotor burst generation in the hemi-spinal cord of the lamprey. *Abstr. Soc. Neurosci*. **65**:61.

Casasnovas, B., and P. Meyrand. 1995. Functional differentiation of adult neural circuits from a single embryonic network. *J. Neurosci.* **15**:5703–5718.

Dale, N., and F.M. Kuenzi. 1997. Ion channels and the control of swimming in the *Xenopus* embryo. *Prog. Neurobiol*. **53**:729–756.

Del Negro, C.A., N. Koshiya, R.J. Butera, Jr., and J.C. Smith. 2002. Persistent sodium current, membrane properties, and bursting behavior of pre-Bötzinger complex inspiratory neurons *in vitro*. *J. Neurophysiol*. **88**:2242–2250.

Dorris, M.C., M. Pare, and D.P. Munoz. 1997. Neuronal activity in monkey superior colliculus related to the initiation of saccadic eye movements. *J. Neurosci*. **17**: 8566–8579.

Duch, C., T. Mentel, and H.-J. Pflüger. 1999. Distribution and activation of different types of octopaminergic DUM neurons in the locust. *J. Comp. Neurol*. **403**:119–134.

Edwards, S.B. 1980. The deep cell layers of the superior colliculus: Their reticular characteristics and structural organization. In: The Reticular Formation Revisited, ed. A. Hobson, and M. Brazier, pp. 193–209. New York: Raven,

Eide, A.L., J. Glover, O. Kjaerulff, and O. Kiehn. 1999. Characterization of commissural interneurons in the lumbar region of the neonatal rat spinal cord. *J. Comp. Neurol*. **403**:332–345.

El Manira, A., P. Kettunen, D. Hess, and P. Krieger. 2002. Metabotropic glutamate receptors provide intrinsic modulation of the lamprey locomotor network. *Brain Res. Brain Res. Rev.* **40**:9–18.

Endo, T., Y. Yanagawa, K. Obata, and T. Isa. 2003. Characteristics of GABAergic neurons in the superficial superior colliculus in mice. *Neurosci. Lett.* **346**:81–84.

Fenelon, V., Y. Le Feuvre, T. Bem, and P. Meyrand. 2003. Maturation of rhythmic neural network: Role of central modulatory inputs. *J. Physiol. (Paris)* **97**:59–68.

Fischer, B., and R. Boch. 1983. Saccadic eye movements after extremely short reaction times in the monkey. *Brain Res*. **260**:21–26.

Frermann, D., B.U. Keller, and D.W. Richter. 1999. Calcium oscillations in rhythmically active respiratory neurones in the brainstem of the mouse. *J. Physiol. Lond.* **515.1**: 119–131.

Goulding, M., G. Lanuza, T. Sapir, and S. Narayan. 2002. The formation of sensorimotor circuits. *Curr. Opin. Neurobiol*. **12**:508–515.

Graham, D. 1985. Pattern and control of walking. *Adv. Insect Physiol*. **18**:31–140.

Graybiel, A.M. 1978a. Organization of the nigrotectal connection: An experimental tracer study in the cat. *Brain Res*. **143**:339–348.

Graybiel, A.M. 1978b. A stereometric pattern of distribution of acetylthiocholinesterase in the deep layers of the superior colliculus. *Nature* **272**:539–541.

Graybiel, A.M., N. Brecha, and H.J. Karten. 1984. Cluster-and-sheet pattern of enkephalin-like immunoreactivity in the superior colliculus of the cat. *Neuroscience* **12**: 191–214.

Grillner, S. 2003. The motor infrastructure: From ion channels to neuronal networks. *Nat. Rev. Neurosci*. **4**:573–586.

Harris-Warrick, R.M., and E. Marder. 1991. Modulation of neural networks for behavior. *Ann. Rev. Neurosci*. **14**:39–57.

Heckman, C.J., R.H. Lee, and R.M. Brownstone. 2003. Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. *TINS* **26**: 688–695.

Hedwig, B., and, K. Pearson. 1984. Patterns of synaptic drive to identified flight motoneurons in the locust. *J. Comp. Physiol*. **154**:745–760.

Hess, D., and A. Büschges. 1999. Role of proprioceptive signals from an insect femur-tibia joint in patterning motoneuronal activity of an adjacent leg joint. *J. Neurophysiol.* **81**:1856–1865.

Hilaire, G., and B. Duron. 1999. Maturation of the mammalian respiratory system. *Physiol. Rev.* **79**:325–360.

Hu, G.Y., Z. Biro, R. H. Hill, and S. Grillner. 2002. Intracellular QX-314 causes depression of membrane potential oscillations in lamprey spinal neurons during fictive locomotion. *J. Neurophysiol*. **87**:2676–2683.

Hultborn, H., B.A. Conway, J.P. Gossard et al. 1998. How do we approach the locomotor network in the mammalian spinal cord? *Ann. NY Acad. Sci*. **860**:70–82.

Illing, R.-B. 1992. Association of efferent neurons to the compartmental architecture of the superior colliculus. *PNAS* **89**:10,900–10,904.

Illing, R.-B., and Graybiel, A.M. 1985. Convergence of afferents from frontal cortex and substantia nigra onto acetylcholinesterase-rich patches of the cat's superior colliculus. *Neuroscience* **14**:455–482.

Isa, T. 2002. Intrinsic processing in the mammalian superior colliculus. *Curr. Opin. Neurobiol.* **12**:668–677.

Isa, T., T. Endo, and Y. Saito. 1998. The visuo-motor pathway in the local circuit of the rat superior colliculus. *J. Neurosci*. **18**:8496–8504.

Jacobs, B.L., and C.A. Fornal. 1997. Serotonin and motor activity. *Curr. Opin. Neurobiol*. **7**:820–825.

Jankowska, E., I. Hammar, U. Slawinska, K. Maleszak, and S.A. Edgley. 2003. Neuronal basis of crossed actions from the reticular formation on feline hindlimb motoneurons. *J. Neurosci*. **23**:1867–1878.

Jessell, T.M. 2000. Neuronal specification in the spinal cord: Inductive signals and transcriptional codes. *Nat. Rev. Genet*. **1**:20–29.

Katz, P.S., and R.M. Harris-Warrick. 1999. The evolution of neuronal circuits underlying species-specific behavior. *Curr. Opin. Neurobiol*. **9**:628–633.

Keller, E.L. 1979. Colliculoreticular organization in the oculomotor system. In: Reflex Control of Posture and Movement, ed. R. Granit and O. Pompeiano, pp. 725–734. Amsterdam: Elsevier.

Kettunen, P., D. Hess, and A. El Manira. 2003. mGluR1, but not mGluR5, mediates depolarization of spinal cord neurons by blocking a leak current. *J. Neurophysiol*. **90**:2341–2348.

Kiehn, O., and, S.J. Butt. 2003. Physiological, anatomical, and genetic identification of CPG neurons in the developing mammalian spinal cord. *Prog. Neurobiol*. **70**: 347–361.

Kiehn, O., and T. Eken. 1998. Functional role of plateau potentials in vertebrate motor neurons. *Curr. Opin. Neurobiol*. **8**:746–752.

Kiehn, O., J. Hounsgaard, and K.T. Sillar. 1997. Basic buildings blocks of vertebrate central pattern generators. In: Neurons, Networks, and Motor Behavior, ed. P.S.G. Stein, S. Grillner, A. Selverston, and D.G. Stuart, pp. 47–59. Cambridge, MA: MIT Press.

Kiehn, O., and K. Kullander. 2004. Central pattern generators deciphered by molecular genetics. *Neuron* **41**:317–321.

Kobayashi, Y., Y. Inoue, M. Yamamoto, T. Isa, and H. Aizawa. 2002. Contribution of pedunculopontine tegmental nucleus neurons to performance of visually guided saccade tasks in monkeys. *J. Neurophysiol*. **88**:715–731.

Koshiya, N., and J.C. Smith. 1999. Neuronal pacemaker for breathing visualized *in vitro*. *Nature* **400**:360–363.

Kudo, N., and T. Yamada. 1987. N-methyl-D,L-aspartate-induced locomotor activity in a spinal cord-hindlimb muscles preparation of the newborn rat studied *in vitro*. *Neurosci. Lett*. **75**:43–48.

Lalley, P.M., A.M. Bischoff, S.W. Schwarzacher, and D.W. Richter. 1995. 5-HT2 receptor-controlled modulation of medullary respiratory neurones in the cat. *J. Physiol*. **487.3**:653–661.

Lee, C., W.H. Rohrer, and D.L. Sparks. 1988. Population coding of saccadic eye movements by neurons in the superior colliculus. *Nature* **332**:357–360.

Lee, P.H., M.C. Helms, G.J. Augustine, and W.C. Hall. 1997. Role of intrinsic synaptic circuitry in collicular sensorimotor integration. *PNAS* **94**:13,299–13,304.

Lee, S.K., and S.L. Pfaff. 2001. Transcriptional networks regulating neuronal identity in the developing spinal cord. *Nat. Neurosci*. **4 Suppl**:1183–1191.

Ma, T.P., Graybiel, A.M., and Wurtz, R.H. 1991. Location of saccade-related neurons in the macaque superior colliculus. *Exp. Brain Res.* **85**:21–35.

Manzke, T., U. Guenther, E.G. Ponimaskin et al. 2003. 5-HT4a receptors avert opioid-induced breathing depression without loss of analgesia. *Science* **301**:226–229.

Marder, E., and R.L. Calabrese. 1996. Principles of rhythmic motor pattern generation. *Physiol. Rev.* **76**:687–717.

Mays, L.E., and D.L. Sparks. 1980. Dissociation of visual and saccade-related responses in superior colliculus neurons. *J. Neurophysiol*. **43**:207–232.

McDearmid, J.R., J.F. Scrymgeour-Wedderburn, and K.T. Sillar. 1997. Aminergic modulation of glycine release in a spinal network controlling swimming in *Xenopus laevis*. *J. Physiol*. **503.1**:111–117.

McLean, D.L., S.D. Merrywest, and K.T. Sillar. 2000. The development of neuromodulatory systems and the maturation of motor patterns in amphibian tadpoles. *Brain Res. Bull.* **53**:595–603.

Mentel, T., C. Duch, H. Stypa et al. 2003. Central modulatory neurons control fuel selection in flight muscle of migratory locust. *J. Neurosci.* **23**:1109–1113.

Mironov, S.L., and D.W. Richter. 1998. L-type Ca^{2+} channels in inspiratory neurones of mice and their modulation by hypoxia. *J. Physiol. Lond.* **512.1**:75–87.

Nishimaru, H., M. Iizuka, S. Ozaki, and N. Kudo. 1996. Spontaneous motoneuronal activity mediated by glycine and GABA in the spinal cord of rat fetuses *in vitro*. *J. Physiol.* **497.1**:131–143.

Nishimaru, H., and N. Kudo. 2000. Formation of the central pattern generator for locomotion in the rat and mouse. *Brain Res. Bull.* **53**:661–669.

Orlovsky, G.N., T. Deliagina, and S. Grillner. 1999. Neuronal Control of Locomotion. From Mollusc to Man. Oxford: Oxford Univ. Press.

Pierrefiche, O., J. Champagnat, and D.W. Richter. 1995. Calcium-dependent conductances control neurones involved in termination of inspiration in cats. *Neurosci. Lett.* **184**:101–104.

Pierrefiche, O., A. Haji, A. Bischoff, and D.W. Richter. 1999. Calcium currents in respiratory neurons of the cat *in vivo*. *Eur. J. Physiol.* **438**:817–826.

Ponimaskin, E.G., J. Profirovic, R. Vaiskunaite, D.W. Richter, and T.A. Voyno-Yasenetskaya. 2002. 5-Hydroxytryptamine 4a receptor is coupled to the Gá subunit of heterotrimeric G13 protein. *J. Biol. Chem*. **277**:20,812–20,819.

Pratt, C.A., and L.M. Jordan. 1987. Ia inhibitory interneurons and Renshaw cells as contributors to the spinal mechanisms of fictive locomotion. *J. Neurophysiol*. **57**:56–71.

Ramirez, J.M., and D.W. Richter. 1996. The neuronal mechanisms of respiratory rhythm generation. *Curr. Opin. Neurobiol*. **6**:817–825.

Richter, D.W., T. Manzke, B. Wilken, and E. Ponimaskin. 2003. Serotonin receptors: Guardians of stable breathing. *Trends Mol. Med.* **9**:542–548.

Richter, D.W., P. Schmidt-Garcon, O. Pierrefiche, A.M. Bischoff, and P.M. Lalley. 1999. Neurotransmitters and neuromodulators controlling the hypoxic respiratory response in anaesthetized cats. J. *Physiol*. **514 Pt 2**:567–578.

Richter, D.W., and K.M. Spyer. 2001. Studying rhythmogenesis of breathing: Comparison of *in vivo* and *in vitro* models. *TINS* **24**:464–472.

Roberts, A., S.R. Soffe, E.S. Wolf, M. Yoshida, and F.Y. Zhao. 1998. Central circuits controlling locomotion in young frog tadpoles. *Ann. NY Acad. Sci*. **860**:19–34.

Robinson, D.A. 1972. Eye movements evoked by collicular stimulation in the alert monkey. *Vision Res*. **12**:1795–1808.

Saito, Y., and T. Isa. 2003. Local excitatory network and NMDA receptor activation generate a synchronous and bursting command from the superior colliculus. *J. Neurosci*. **23**:5854–5864.

Schmidt, J., H. Fischer, and A. Büschges. 2001. Pattern generation for walking and searching movements of a stick insect leg. II. Control of motoneuronal activity. *J. Neurophysiol.* **85**:354–361.

Scudder, C.A., C.S. Kaneko, and A.F. Fuchs. 2002. The brainstem burst generator for saccadic eye movements: A modern synthesis. *Exp. Brain Res*. **142**:439–462.

Sharma, K., and C.Y. Peng. 2001. Spinal motor circuits: Merging development and function. *Neuron* **29**:321–324.

Sillar, K.T., O. Kiehn, and N. Kudo. 1997. Chemical modulation of vertebrate motor circuits. In: Neurons, Networks, and Motor Behavior, ed. P.S.G. Stein, S. Grillner, A. Selverston, and D.G. Stuart, pp. 183–193. Cambridge, MA: MIT Press.

Smith, J. 1997. Integration of cellular and network mechanisms in mammalian oscillatory motor circuits: Insights from the respiratory oscillator. In: Neurons, Networks, and Motor Behavior, ed. P. S.G. Stein, S. Grillner, A. Selverston, and D. Stuart, pp. 97–104 Cambridge, MA: MIT Press.

Smith, J.C., R.J. Butera, N. Koshiya, C. Del Negro, C.G. Wilson, and S.M. Johnson. 2000. Respiratory rhythm generation in neonatal and adult mammals: The hybrid pacemaker-network model. *Respir. Physiol*. **122**:131–147.

Smith, J.C., H. Ellenberger, K. Ballanyi, D.W. Richter, and J.L. Feldman. 1991. Pre-Bötzinger complex: A brainstem region that may generate respiratory rhythm in mammals. *Science* **254**:726–729.

Sombati, S., and G. Hoyle. 1984. Generation of specific behaviors in a locust by local release into neuropil of the natural neuromodulator octopamine. *J. Neurobiol*. **15**: 481–506.

Sparks, D.L. 1978. Functional properties of neurons in the monkey superior colliculus: Coupling of neuronal activity and saccade onset. *Brain Res*. **156**:1–16.

Sparks, D.L. 2002. The brainstem control of saccadic eye movements. *Nat. Rev. Neurosci*. **3**:952–964.

Sparks, D., and R. Hartwich-Young. 1989. The deeper layers of the superior colliculus. In: The Neurobiology of Saccadic Eye Movements, ed. R. Wurtz and M. Goldberg, pp. 213–255. Amsterdam: Elsevier.

Sparks, D., W.H. Rohrer, and Y. Zhang. 2000. The role of the superior colliculus in saccade initiation: A study of express saccades and the gap effect. *Vision Res*. **40**:2763–2777.

Stokke, M.F., U.V. Nissen, J.C. Glover, and O. Kiehn. 2002. Projection patterns of commissural interneurons in the lumbar spinal cord of the neonatal rat. *J. Comp. Neurol*. **446**:349–359.

Wallen, P., and S. Grillner 1987. N-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. *J. Neurosci.* **7**:2745–2755.

Zhang, W., F. Elsen, A. Barnbrock, and D.W. Richter. 1999. Postnatal development of $GABA_B$ receptor-mediated modulation of voltage-activated Ca^{2+} currents in mouse brain-stem neurons. *Eur. J. Neurosci.* **11**:2332–2342.

6

Microcircuits in the Striatum

Cell Types, Intrinsic Membrane Properties, and Neuromodulation

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ABSTRACT

The principal neurons of the striatum are GABAergic medium spiny neurons. Approximately 90% of all striatal neurons fall into this anatomical class (at least in the rodent). Medium spiny neurons are commonly subdivided on the basis of axonal projections into striatopallidal and striatonigral subclasses, which are often referred to as the "indirect" and "direct" pathways (Albin et al. 1989). In addition to medium spiny projection neurons, there are at least four types of striatal interneurons (Kawaguchi et al. 1995). The best studied of these (because of its size and autonomous activity) is the giant aspiny cholinergic interneuron. The other three types are nominally GABAergic: (1) fast-spiking, parvalbumin-expressing interneurons, (2) somatostatin/neuropeptide Y/nitric oxide synthase (NOS)-expressing interneurons, and (3) calretinin-expressing interneurons.

This chapter focuses initially on basic electrophysiological and neuromodulatory mechanisms of medium spiny neurons and cholinergic interneurons—the two cell types about which most is known. Questions addressed are:

- To what extent are UP state transitions and spiking in medium spiny neurons influenced by intrinsic ionic mechanisms subject to neuromodulation by dopamine/acetylcholine?
- Previous work has shown that spike generation during the UP state in medium spiny neurons is governed by processes that appear to be independent of those controlling the UP state transition itself. What are these processes? Could they act to gate sensorimotor signals? If so, is this process controlled by dopamine/acetylcholine? Is it controlled by ongoing activity?
- How does dopamine regulate the activity of cholinergic interneurons?
- How might task-related changes in intrastriatal dopamine levels alter the function of microcircuits composed of cholinergic interneurons and medium spiny neurons of the so-called direct and indirect pathways?

IONIC MECHANISMS UNDERLYING STATE TRANSITIONS IN MEDIUM SPINY NEURONS

In this section I will attempt to summarize what is known about the intrinsic ionic mechanisms controlling synaptically driven state transitions in medium spiny neurons. These "naturally occurring" transitions have been studied primarily with conventional sharp electrode techniques in anesthetized rodents. Wilson and colleagues have made a compelling case that these synaptically driven phenomena are critical to understanding the way in which medium spiny neurons integrate synaptic information and communicate with striatal and extrastriatal modules. The portrait of these phenomena has been painted here with broad strokes as there are significant gaps in our understanding of the molecular mechanisms underlying these transitions. The treatment leans heavily toward a basic ionic mechanism with the assumption that this approach is the best strategy to achieve an unbiased reconstruction of the unmodulated and modulated behavior of striatal neurons. This approach has been successful in unraveling the actions of dopamine, acetylcholine, and other modulators working through G-protein-coupled receptors (GPCRs) when conventional approaches have failed. On the face of it, this failure stems from the inability of conventional approaches that rely upon tissue slices or *in vivo* recording from anesthetized animals to recreate key neuronal states that are targeted by GPCR cascades. The more reductionist approaches rely less on the recreation of these states but generate fragmentary information about what GPCRs are doing. Weaving these fragments into the whole cloth of the integrated response to dopamine and acetylcholine is beginning.

THE DOWN STATE

In the absence of synaptic input, the somatic membrane potential of medium spiny neurons is stable near the K^+ equilibrium potential (Wilson 1993). This is the so-called "DOWN state" of medium spiny neurons. Residence in the DOWN state is attributable to inwardly rectifying K^+ currents (Uchimura et al. 1989; Wilson 1993; Mermelstein et al. 1998). These currents flow through constitutively open Kir2 (IRK) family channels, which block at membrane potentials above the K^+ equilibrium potential as Mg^{2+} and polyamines are swept into the pore. In medium spiny neurons, this rectification is strong as expected of Kir2 channels (Figure 6.1a). These channels are multimeric and thought to consist of four pore-forming subunits. Four subunits have been cloned; three of which are expressed at high levels in the striatum (Kir2.1–3). Interestingly, striatopallidal and striatonigral neurons appear to differ in their expression of these subunits (Figure 6.1b–c). Both cell types express high levels of Kir2.2 mRNA; however, enkephalin (ENK)-expressing striatopallidal neurons largely co-express Kir2.1 subunits, whereas substance P (SP)-expressing striatonigral

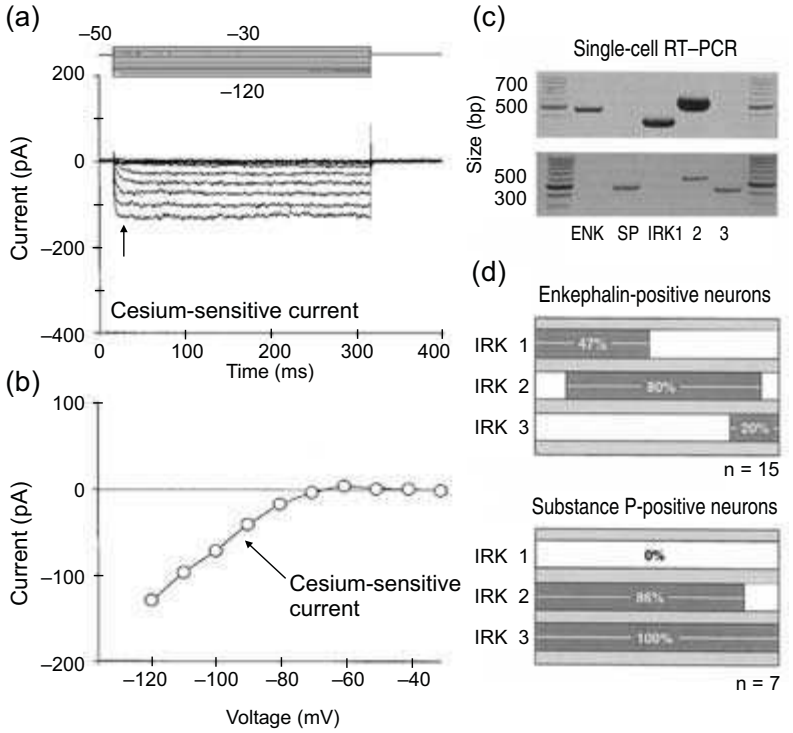

Figure 6.1 Inwardly rectifying K^+ currents in medium spiny neurons are attributable to Kir2 channels. (a) Cs^+-sensitive inwardly rectifying K^+ currents in medium spiny neurons evoked by a series of voltage steps (–30 mV to –120 mV in 10 mV increments) from –50 mV (E_K). (b) Current/voltage relationship of the inwardly rectifying K^+ current, measured at the arrow in (a). (c) Single-cell RT-PCR showing the correlation of IRK (Kir2) subunit expression with ENK and SP expression. The ENK mRNA-positive neuron (top) expressed detectable levels of IRK1 (Kir2.1) and IRK2 (Kir2.2). On the other hand, the SP-positive neuron (bottom) expressed IRK2 (Kir2.2) and IRK3 (Kir2.3). (d) Summarized data comparing IRK mRNA subunit expression in ENK-positive (n = 15) and SP-positive (n = 7) neurons.

neurons co-express Kir2.3 subunits. This difference in subunit expression was correlated with the gating of channels in that currents in striatopallidal neurons declined (10–20%) with maintained hyperpolarization. This property of the channels should lead to a time-dependent increase in the excitability of striatopallidal neurons with residence in the DOWN state. This time-dependent change in gating may reflect voltage-dependent alterations in the interaction of PIP2 with the Kir2 channels (Soom et al. 2001; Xiao et al. 2003); PIP2 binding to the Kir2 subunits is necessary for channel opening. Differences in the affinity of the Kir2 subunits for PIP2 could also be a determinant in their susceptibility to modulation by GPCR activation of PLC isoforms that deplete the membrane of PIP2.

Kir2 channels are important in regulating synaptic integration as well as the resting membrane potential. Wilson (1993) has shown that inwardly rectifying currents shape the response to intracellular current injection in several ways. First, as somatic and dendritic Kir2 channels block with membrane depolarization, the membrane time constant is increased, slowing the voltage response. This leads to a ramp-like voltage trajectory of medium spiny neurons with step current pulses (without the introduction of depolarization-activated K^+ channels). Although there are other channels that are activated by depolarization that contribute to this phenomenon in medium spiny neurons, Kir2 channels are clearly important. Second, as Kir2 channels close with membrane depolarization, the effective electrotonic structure of medium spiny neurons changes; closure of the Kir2 channels makes the dendrites more compact, bringing excitatory synaptic inputs effectively closer to the soma. This alteration in the electrotonic structure with depolarizing synaptic input has been advanced as an explanation for the rather abrupt and stereotyped transition between DOWN and UP states seen in medium spiny neurons.

THE TRANSITION TO AND MAINTENANCE OF THE UP STATE

The transition to the UP state in medium spiny neurons is driven by cortical excitatory synaptic input. The evidence for this conclusion is beautifully summarized by Wilson and Kawaguchi (1996). As the authors demonstrate, the late stages of the transition and the membrane potential of the UP state itself are dependent upon K^+ channels that are blocked by intracellular Cs^+. There are at least three kinetically distinct K^+ channels that begin to activate in the voltage range of the UP state (ca. –50 mV), which may contribute to this dependence. These have traditionally been referred to as the fast and slow A-type currents and the persistent current (Nisenbaum et al. 1994; Nisenbaum and Wilson 1995). The fast A-type K^+ current is attributable to Kv4 channels (Tkatch et al. 2000). These channels are present at relatively low densities in the somatic membrane, and there is some evidence for heterogeneity within the medium spiny neuron population (Tkatch et al. 2000). As with other K^+ channels, Kv4 channels are thought to be tetrameric; Kv4.2 subunits appear to make the largest contribution to medium spiny neurons channels, but Kv4.1 and Kv4.3 subunits also contribute. There are undoubtedly other (as yet unidentified) modifying subunits (e.g., KChips, DPXX) that further shape gating of these channels as they activate and inactivate at more depolarized potentials than Kv4 currents in a variety of other cell types.

The identity of the slow A-type current has been something of a mystery until recently, when it was shown that it was attributable to Kv1.2 channels (Shen et al. 2004). These channels activate (and inactivate) more slowly than Kv4 channels and are selectively blocked by dendrotoxin. They also recover from

inactivation very slowly, creating a form of activity-dependent plasticity. The current through these channels is a major component (ca. 50%) of the K^+ current active in the subthreshold voltage range of the UP state in medium spiny neurons. Block of Kv1.2 channels speeds the rate at which current injection depolarizes medium spiny neurons, shortening first the spike latency and increasing discharge frequency (Figure 6.2). This acceleration is due to the participation of Kv1.2 channels in the afterhyperpolarization of medium spiny neurons.

The persistent K^+ current activates more slowly and does not inactivate (or does so very slowly). Recent work has revealed that this is largely attributable to a KCNQ2/3 channel (M-channel), which is exquisitely sensitive to

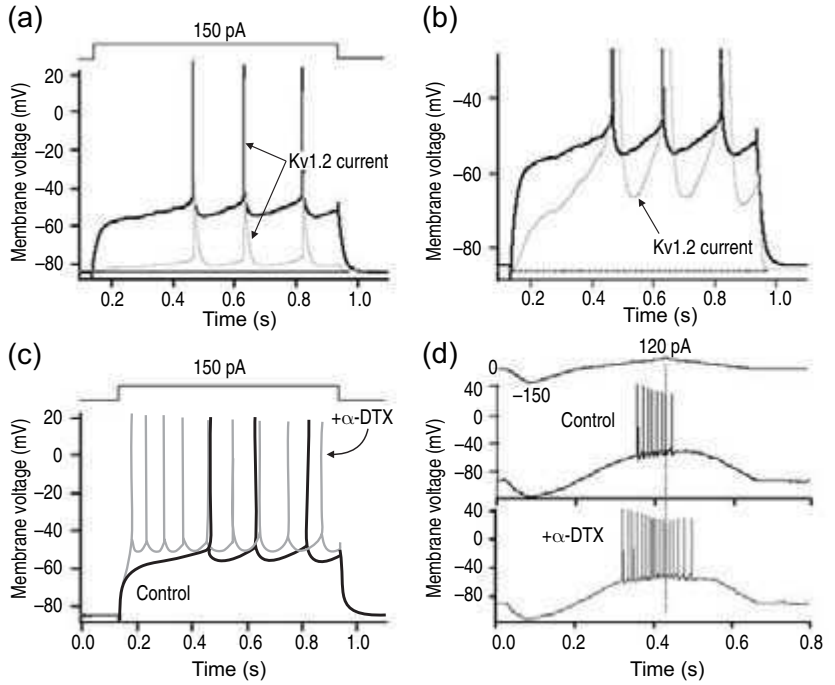

Figure 6.2 Kv1.2 channels prolong first spike latency and slow repetitive discharge. (a) The black line shows the voltage response of a medium spiny neuron recorded in a slice to a near-rheobase current injection. The gray line denotes the simulated Kv1.2 current evoked by the same voltage trajectory. Note that the Kv1.2 current deactivated slowly and remained activated during inter-spike period. (b) The same data as in (a) at higher gain to illustrate the Kv1.2 channel currents during the inter-spike period. (c) After bath application of 1 μM α-DTX, the first spike latency of the medium spiny neuron shown in (a) was shortened, the fast afterhyperpolarization reduced, and the repetitive discharge rate increased, as suggested by the Kv1.2 model. (d) Action potentials (APs) evoked by a symmetric 4 second long current ramp shown at the top of (d) in the same neuron before and after application of α-DTX (1 μM). Note that α-DTX shortened the latency to the first AP and increased the repetitive firing.

acetylcholine (Shen et al. 2004). Although they activate in this subthreshold voltage range, KCNQ channels differ in two important respects from Kv4 and Kv1.2 channels: (a) they do not inactivate, and (b) they activate (and deactivate) much more slowly than these other channels. Blockade of KCNQ channels depolarizes neurons held at UP state potentials and accelerates the discharge in medium spiny neurons in response to slow current ramps.

Do these channels contribute to regulation of UP state spiking in medium spiny neurons? As originally demonstrated by Wilson and Kawaguchi (1996), blockade of K^+ channels with Cs^+ has an enormous impact on the UP state potential. This led them to suggest that K^+ channels were the key intrinsic factor governing the UP state membrane potential distribution. Subsequently, Wickens and Wilson (1998) have suggested that the difference between medium spiny neurons that spike and those that do not during UP state transitions *in vivo* (anesthetized rats) is that the UP state membrane potential of those that do is a few millivolts more positive than those that do not. Given the importance of Kv1.2 and KCNQ channels in this voltage range, it is easy to speculate that the modulation state of either channel (see below) could be responsible for the difference between spiking and not.

Do channels that give rise to inward currents play a role in the UP state? As the membrane potential moves above ca. –65 mV, voltage-dependent Na^+ channels begin to activate providing an inward, depolarizing current that will speed the membrane potential movement toward the UP state plateau near –55 mV. These currents are attributable to a mix of Na^+ channels with pore-forming subunits of the Nav1.1, Nav1.2, and Nav1.6 classes. Channels with these subunits give rise to both persistent and transient currents. However, the relative contribution of each channel to each component of the macroscopic current may vary from one cell type to another for as yet unknown reasons. For example, in cortical pyramidal neurons, roughly two thirds of the persistent Na^+ current seen in somatic/proximal dendritic recordings is attributable to Nav1.6 channels. This may turn out to be significant for several reasons. One is that the subcellular distribution of these channel types does not appear to be uniform (Gong et al. 1999; Surmeier, unpublished). Nav1.1 channels are largely somatic, whereas Nav1.2 and Nav1.6 channels are more widely distributed. Another potentially important factor is phosphoregulation. Nav1.6 channels lack critical protein kinase A (PKA) phosphorylation sites, making them insensitive to modulation by this protein kinase, which is a major effector of D_1 receptor signaling. D_2 and M_1 muscarinic receptor stimulation of protein kinase C (PKC), on the other hand, should modulate all forms of the Na^+ channel alpha subunit.

There is no question that voltage-dependent Na^+ channels shape the membrane potential trajectory in response to depolarizing current injection delivered to the soma, but do they contribute to the UP state transition and maintenance of the UP state? Wilson and Kawaguchi (1996) claim not. They found that UP states recorded in an urethane-anesthetized rat were unaffected by somatic

loading of QX-314 through a sharp electrode. QX-314 blocks Na^+ channels at sufficiently high concentrations, although not selectively. This result is a bit perplexing and is now being challenged by studies in *in vitro* preparations. In organotypic slice cultures, Na^+ channels in dendritic regions of medium spiny neurons are capable of supporting backpropagating spikes well into distal regions (Kerr and Plenz 2002), suggesting that orthodromically initiated events should be capable of generating slow regenerative events in dendrites (as in cortical pyramidal neurons). In corticostriatal slices, state transitions resembling those seen *in vivo* can be produced under certain conditions (Vergara et al. 2003). An example of these transitions is shown in Figure 6.3. Surprisingly, the medium spiny neuron membrane potential distributions found in this preparation are bimodal and strongly resemble those found in anesthetized rodents. For comparison, a histogram taken from *in vivo* work by Wickens and Wilson (1998) is shown alongside that taken from the slice preparation.

In contrast to what has been found *in vivo*, plateau potentials (UP states) generated in the slice preparation are blocked by QX-314 delivered with a somatic patch pipette (unpublished observations). There are two obvious ways in which the differences between the *in vitro* and *in vivo* results can be explained. One is that because QX-314 was delivered with a sharp electrode, it was not present at high enough concentrations in the dendritic region to block Na^+ channels effectively in the Wilson and Kawaguchi experiments. Another is that the mechanisms governing dendritic electrogenesis in the slice and in the anesthetized rat are different. This difference could stem from a GPCR-mediated suppression of dendritic Na^+ channel opening in anesthetized animals, eliminating them as a significant factor in the response to synaptic stimulation and dendritic electrogenesis. Another possibility is that the degree of afferent synchrony achieved in the anesthetized preparation masks any contribution of intrinsic mechanisms to dendritic electrogenesis that might occur in other circumstances.

Another depolarizing influence at voltages near the UP state stems from voltage-activated Ca^{2+} channels. Mature medium spiny neurons express Cav1.2, Cav1.3, Cav2.1, Cav2.2, and Cav2.3 voltage-activated Ca^{2+} channels. The Cav2 family channels (N-, P/Q- and R-types) are activated only at membrane potentials achieved during spiking. Ca^{2+} entry through these channels during spiking shapes repetitive activity by opening nearby Ca^{2+}-dependent K^+ channels of the SK class (Vilchis et al. 2000). Another high voltage-activated Ca^{2+} channel opened only by spiking is the L-type Cav1.2 (C-type) channel. This channel is commonly a sensor of somatic spiking that helps control activity-dependent gene transcription (Bading et al. 1993). Medium spiny neurons express high levels of another L-type Ca^{2+} channel (Cav1.3 L-type Ca^{2+} channels) that activate at significantly more negative membrane potentials (ca. 15 mV) than do the other Ca^{2+} channels. Recent work suggests that these channels are preferentially located at excitatory synaptic sites in medium spiny neurons. Cav1.3 channels appear to interact with the synaptically targeted scaffolding protein Shank

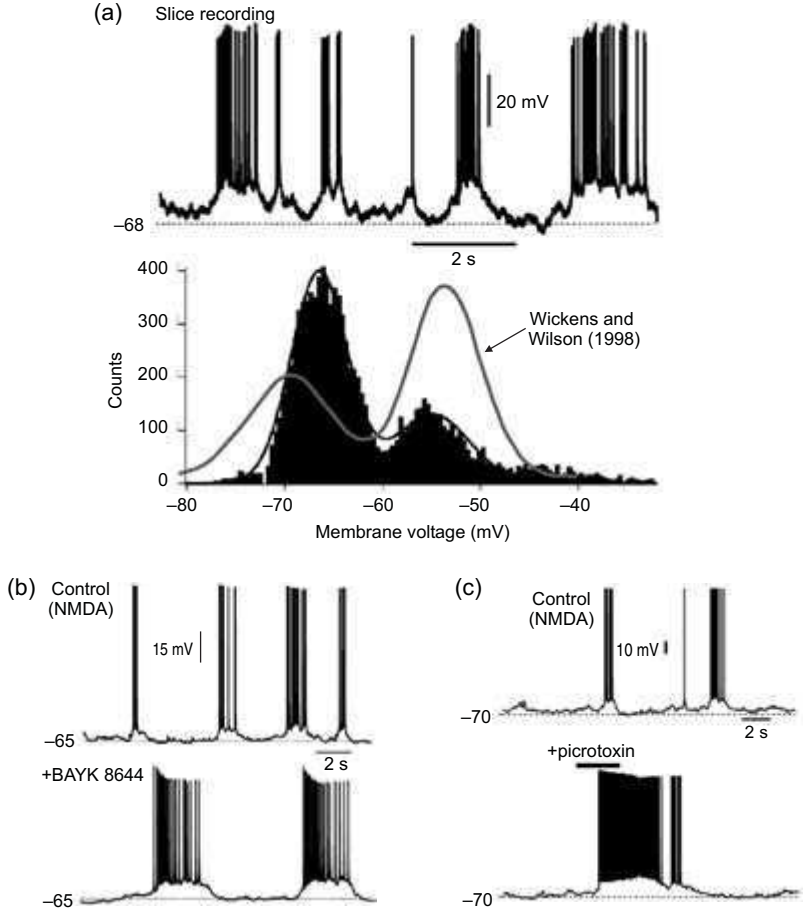

Figure 6.3 In the slice preparation, medium spiny neurons can display state transitions resembling those seen *in vivo*. (a) Patch clamp recording from a medium spiny neuron in a slice preparation after repetitive stimulation of the cerebral cortex. An all points histogram of the membrane potential in this record is shown in the bottom panel. Note the bimodal distribution of membrane potential. The gray line indicates the distribution of a medium spiny neuron seen *in vivo* during state transitions, as described by Wickens and Wilson (1998). (b) Bath application of NMDA (10–15 μM) can substitute for cortical stimulation in producing state transitions in a subset of medium spiny neurons (top panel). The UP state duration is increased by co-application of the L-type channel agonist BAYK 8644 (1 μM) (bottom panel). (c) The duration of NMDA-induced UP state transitions can be increased through coincident application of the $GABA_A$ receptor antagonist picrotoxin (1 μM), suggesting that GABAergic input may regulate the duration of the UP state.

(Sheng and Kim 2000), forming a component of a macromolecular signaling complex at the postsynaptic density involving glutamate receptors, Homer, IP3 receptors, and protein phosphatases (see below). Since the vast majority of the

excitatory synapses in medium spiny neurons are on the heads of dendritic spines (Bolam et al. 2000), Cav1.3 channels are perfectly positioned to augment the depolarization induced by glutamate receptor activation. Because the membrane potentials achieved in the spine are likely to be significantly more depolarized than in the parent dendrite and soma, these channels should readily be engaged and contribute to maintaining membrane potentials necessary for NMDA receptor opening. This co-localization helps explain the commonly reported synergism between L-type channels and NMDA receptor signaling (e.g., Konradi et al. 1996; Cepeda et al. 1998). Since Cav1.3 channels are potently regulated by GPCRs activated by dopamine and acetylcholine (see below), their contribution to boosting of glutamatergic currents responsible for the UP state transition is subject to regulation.

REPETITIVE SPIKING IN THE UP STATE

Medium spiny neurons are typically called regular-spiking neurons. That is, they are neurons with modest maximal discharge rates, relatively little spike frequency adaptation (wide primary range), and modest spike accommodation. The Na^+ channel currents expressed by medium spiny neurons are not unusual in that there are transient and persistent current components; they do not express resurgent Na^+ current. The voltage dependence of activation and fast inactivation resembles that of cortical pyramidal neurons. In the soma/initial segment region, these currents are likely to be dominated by Nav1.1 channels, whereas in the dendritic regions, Nav1.2- and Nav1.6-containing channels are more likely to dominate. This differential subcellular distribution could be a significant factor in determining the electrogenesis and the impact of neuromodulators (e.g., Nav1.6 channels tend to gate in the persistent mode and are refractory to PKA regulation). As described above, Kv4, Kv1.2, and KCNQ K^+ channel currents and Cav1.3 Ca^{2+} channel currents are active in the subthreshold range and will interact with Na^+ channel currents in determining spike threshold (ca. –45 mV).

Spike repolarization appears to be handled largely by Kv2 and BK channels with minor contributions from Kv1.2 and Kv4 channels. As a consequence of this reliance upon slowly activating conductances, spike width is relatively broad (1.6–2.0 ms). Spike width is an important feature for a variety of reasons. Perhaps the best-studied consequence is the regulation of Ca^{2+} entry through high voltage-activated Ca^{2+} channels during the spike; the duration of the spike has a significant impact on the magnitude of entry.

Spike afterhyperpolarization largely arises from the slow deactivation of Kv2 and Kv1.2 channels and the activation of calcium-dependent K^+ channels (SK). SK channels are controlled by Ca^{2+} flux through Cav2.1/2.2 channels turned on during the spike (Vilchis et al. 2000). However, SKs make a less prominent contribution to repetitive discharge in medium spiny neurons than in some other regular-spiking neurons (e.g., pyramidal neurons) as judged from the lack of prominent spike frequency adaptation.

TERMINATION OF THE UP STATE

Relatively little is known about termination of the UP state. The prevailing view, based largely upon the work of Wilson (1993), is that this is a passive event reflecting the reemergence of DOWN states in cortical pyramidal neurons projecting to the striatum. That is, at some point in time the glutamatergic drive drops below that necessary to maintain the UP state, leading to movement of the medium spiny neuron membrane potential to the stable point defined by Kir2 channel activity. There is little doubt that this is a major part of the story, particularly in anesthetized animals. However, in the slice preparation where plateau potentials have been recreated by generating sustained cortical activity, DOWN state transitions still occur. This situation may more closely resemble the situation in the awake animal, where cortical DOWN state transitions appear to be much briefer or nonexistent (Bazhenov et al. 2002). Furthermore, disruption of $GABA_A$ synaptic input significantly lengthens plateau duration (Vergara et al. 2003) (Figure 6.3C). Both observations point to more active mechanisms, like feedforward inhibition from FS interneurons, in the regulation of the DOWN state transition. Once the transition has occurred, its maintenance could be due to disfacilitation, as suggested by Wilson and colleagues, particularly if maintenance of the UP state is dependent in some measure upon intrinsic mechanisms.

INTRINSIC IONIC MECHANISMS UNDERLYING ACTIVITY PATTERNS IN CHOLINERGIC INTERNEURONS

Cholinergic interneurons are autonomous pacemakers: they are capable of maintained spike discharge (<5 Hz) in the absence of synaptic input (Bennett et al. 2000). Pacemaking is dependent upon voltage-dependent Na^+ channels possessing Nav1.1, Nav1.2, and Nav1.6 alpha subunits and Navbeta1–3 auxiliary subunit (Maurice et al. 2004). Unlike channels in regular-spiking neurons, the Na^+ channels in cholinergic interneurons have relatively depolarized fast inactivation voltage dependence. The half-inactivation voltage is 8–10 mV more depolarized in interneurons than in neighboring, regular-spiking medium spiny neurons or cortical pyramidal neurons. At their modal membrane potential near –55 mV, roughly half of the Na^+ channels in cholinergic interneurons are inactivated at steady state, in contrast to roughly 80% in medium spiny neurons (or cortical pyramidal neurons) at the same membrane potential.

Perhaps as significant to maintaining Na^+ channel availability and pacemaking is the relatively low probability that interneuronal Na^+ channels reside in the slow inactivated state. For example, holding the membrane potential at –20 mV for 5 seconds led to the slow inactivation of only about 20% of the available channel population. This stands in sharp contrast to the situation in cortical pyramidal neurons, where the same protocol moves roughly 60% of the

channels into this unavailable state. At –60 mV (near the modal membrane potential during single-spike pacemaking), less than 10% of the Na^+ channel population appears to be in the slow inactivated state, and this proportion is likely to be smaller at physiological Na^+ concentrations (Townsend and Horn 1997). This distinctive feature of interneuronal Na^+ channels was not an obvious consequence of the rate of entry into the slow inactivated state or the voltage dependence of the process. It may be that basal serine/threonine kinase phosphorylation of Na^+ channels in cholinergic interneurons is low, retarding channel entry into this unavailable state (Carr et al. 2003). This could result from relatively high levels of protein phosphatase activity targeting Na^+ channels or by reliance upon Na^+ channels that have a low probability of being phosphorylated by protein kinases responsible for setting the basal tone.

HCN (HCN1–4) channels, which are expressed at high levels, also contribute to the pacemaking mechanism (Bennett et al. 2000; Surmeier, unpublished). Spikes in these neurons are broad, reflecting the reliance upon Kv4 (Kv4.2/4.3) and BK channels for repolarization. These broad spikes allow substantial Ca^{2+} influx through voltage-dependent channels during the spike. Cav1.2, 1.3, 2.1, 2.2, and 2.3 Ca^{2+} channels are expressed by interneurons. Ca^{2+} flux through an undefined subset of these channels activates apamin-sensitive SK channels leading to a prominent afterhyperpolarization. These currents pace the single-spike discharge mode of cholinergic interneurons helping to keep it slow and rhythmic in the absence of synaptic input. KCNQ channels are also expressed by cholinergic interneurons, but their functional role has not been critically assessed.

Cholinergic interneurons also occasionally discharge in what has been referred to as a rhythmic burst mode (Bennett et al. 2000). In this mode, rhythmically appearing spike clusters are followed by a prolonged hyperpolarization and then a slow ramp up to the next cluster of spikes. Transition into this mode of firing can be produced by blocking SK channels with apamin. This suggests that interneurons are capable of both discharge modes. It is of considerable interest that something resembling this rhythmic burst mode is more commonly observed in dopamine-depleted than in normo-sensitive monkeys (Raz et al. 2001).

DOPAMINERGIC MODULATION OF THE INTRINSIC PROPERTIES OF MEDIUM SPINY NEURONS

Overview of Classical Models

The now "classical" model of dopamine action in the striatum was originally advanced by Albin, Penney, and Young (1989) over a decade ago. In this model, activation of D_1 receptors excites neurons of the "direct" striatonigral pathway,

whereas activation of D_2 receptors inhibits medium spiny neurons of the "indirect" striatopallidal pathway. The evidence for this model came almost entirely from indirect measures of neuronal activity (e.g., alterations in gene expression, glucose utilization). We now know that this model is in error in several fundamental respects. The most significant conceptual error is in treating dopamine as a transmitter of the same ilk as glutamate and GABA; dopamine receptors do not act like classical neurotransmitter receptors mediating fast synaptic transmission. They do not produce excitatory or inhibitory postsynaptic potentials in the usual sense. Rather, they are G-protein-coupled receptors that alter cellular excitability by modulating the gating of voltage-dependent and ligand-gated (ionotropic) ion channels.

This realization has very basic implications for thinking about what dopamine and other neuromodulators do to neuronal function. For example, the application of dopamine to a resting neuron may do nothing obvious. This experiment has been repeated many times, leading many electrophysiologists to conclude that dopamine does little or nothing in the striatum (Nicola and Malenka 1998). As described above, medium spiny neurons have a stable resting membrane potential that is determined by Kir2 channels. Unless activation of dopamine receptors had a profound and consistent effect on these channels, an electrophysiologist operating with this mindset would see no obvious or consistent response to the application of dopamine.

What activation of dopamine receptors does do, however, is to alter the way in which medium spiny neurons respond to synaptic stimulation, either by altering the properties of the ionotropic receptors this stimulation engages or by altering the properties of the intrinsic voltage-dependent channels activated by the induced change in membrane potential. The frequently observed interaction between dopamine and glutamate can be explained simply in these terms. Without glutamate, nothing happens in medium spiny neurons; they sit at the K^+ equilibrium potential and the principal channels targeted by dopamine are not engaged. As described below, voltage clamp work with medium spiny neurons over the past decade has made it clear that both D_1 and D_2 receptors preferentially target ion channels that are activated only during synaptically driven activity: the UP state. Thus, the role of dopamine is clearly to control this state transition. Within this context, a new understanding of basal ganglia function is emerging that should open new arenas of investigation and also encompasses the work upon which the classical model was based.

D_1 Receptor Modulation

Medium spiny neurons in the so-called direct pathway express SP and D_1 receptors at high levels (Graybiel 1986; Gerfen 1992; Surmeier et al. 1996). These receptors are positively coupled to adenylyl cyclase (Type V) through Golf; elevation in cytosolic cAMP levels leads to the activation of PKA. PKA has a variety

of intracellular targets that affect cellular excitability. There are at least three ion channels involved in the regulation of state transitions that are modulated by the D_1/PKA cascade in medium spiny neurons.

L-type Ca^{2+} channel open probability and currents are enhanced by PKA phosphorylation of a carboxyl terminal motif in medium spiny neurons, as in cardiac cells (Surmeier et al. 1995). As described below, there is growing evidence that the principal target of PKA in this cascade is a subtype of L-type channel (Cav1.3a) that is anchored in dendritic spines (Olson et al. 2005). The enhancement of channel open probability is reversed by M_1 muscarinic receptor signaling, which mobilizes calcineurin (PP2B), resulting in channel dephosphorylation. It has recently been discovered that this selective negative regulation of synaptically positioned Cav1.3 Ca^{2+} channels is controlled by another phosphoprotein, termed regulator of calmodulin signaling (RCS) (Rakhilin et al. 2004). Phosphorylation of RCS dramatically increases its affinity for Ca^{2+}-calmodulin (CaM), making it a CaM sink. By regulating the phosphorylation state of RCS, D_1 receptors and PKA can efficiently regulate the phosphorylation state, susceptibility to modulation by D_2 and M_1 receptors, and function of synaptic Cav1.3a channels.

Another target of D_1 receptor signaling is the voltage-dependent Na^+ channel. Both Nav1.6 and Nav1.1 channels have a pronounced tendency to gate in reopening or persistent mode, whereas Nav1.2 channels are less inclined to do this. Although Nav1.1 is efficiently phosphorylated by PKA, Nav1.6 is not. This suggests that D_1 receptor activation will promote the slow inactivation of somatic Na^+ channels but leave dendritic Na^+ channels unaffected (Carr et al. 2003). Given the potential role of dendritic Na^+ channels and persistent Na^+ currents in dendritic electrogenesis, this could be a very significant difference.

In a slice, the induction of UP states by cortical stimulation is facilitated by the exogenous application of D_1 receptor agonists (Figure 6.4a). This is consistent with the role of Cav1.3 channels in the induction of UP state transitions in this preparation and the enhancement of these currents by D_1 receptor signaling. After the induction of UP state transitions, application of D_1 receptor agonists lengthens UP states, as one would expect following enhancement of Cav1.3 channels and the apparent lack of dendritic Na^+ channel modulation. D_1 receptor-mediated enhancement of NMDA receptor currents is consistent with this modulation of voltage-dependent conductances (Cepeda et al. 1993; Levine et al. 1996; Snyder et al. 1998; Flores-Hernandez et al. 2002). Increasing the probability and lengthening the duration of UP states in medium spiny neurons of the direct pathway, in response to cortical excitatory synaptic input, could subserve a variety of functions: (a) it may help maintain activity in cortical-basal ganglionic-thalamic circuits contributing to working memory (Beiser and Houk 1998; (b) it may allow for polymodal integration necessary for proper execution of movements; (c) activity-dependent synaptic plasticity may rely upon synaptic activity being coincident with backpropagating spikes; that is, UP states may

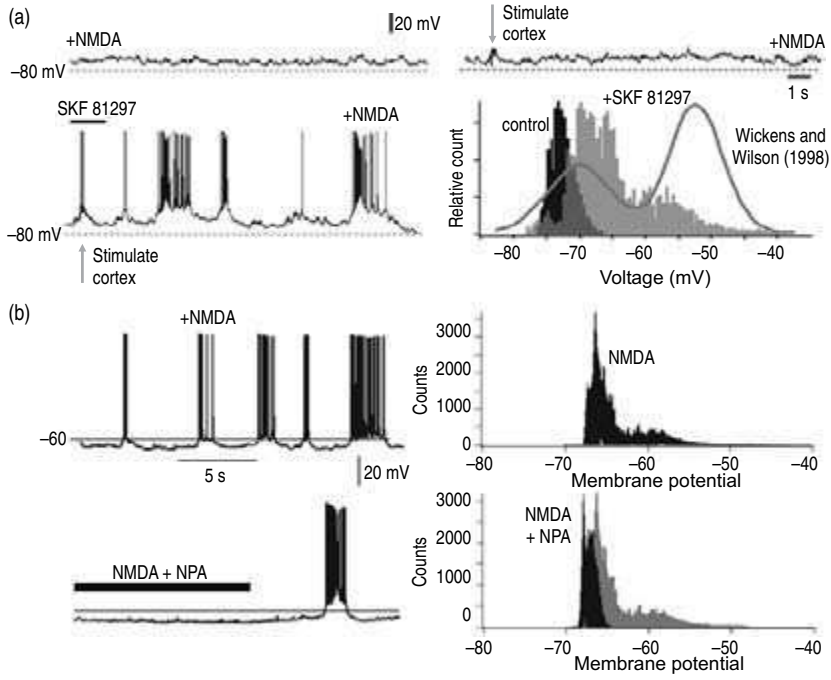

Figure 6.4 Dopamine can regulate state transitions seen in the slice preparation. (a) Top left: Whole-cell recording from a medium spiny neuron in a corticostriatal slice preparation in the presence of NMDA; top right: cortical stimulation in this cell failed to produce UP states. Bottom left: application of the D_1 receptor agonist SKF 81297 (5 μM) prior to stimulation enabled UP states to be produced; bottom right: all points histogram of membrane potential before (black) and after (gray shading) D_1 receptor/cortical stimulation. Gray line is the envelope of the distribution found in Wickens and Wilson (1998). (b) Left: After induction of UP states with NMDA (10 μM), the application of the D_2 receptor agonist NPA (10 μM) suppressed UP states. Right: all points histogram of the membrane potential before NPA application (top) and after (bottom, black). Overlay of the preceding membrane potential distribution is shown in gray.

serve to promote spike backpropagation into the dendritic tree of medium spiny neurons (Kerr and Plenz 2002).

D_2 Receptor Modulation

D_2 receptors and ENK are expressed at high levels in neurons of the striatopallidal or "indirect" pathway. The classical view of this pathway is that D_2 receptors couple to G_i proteins, leading to an inhibition of adenylyl cyclase and an antagonism of D_1 receptor signaling. Aside from the fact that D_1 and D_2 receptors are co-localized in only a small subset of medium spiny neurons, there is

another fundamental problem with this model. First, D_2 receptors do not couple efficiently to G_i proteins (Jiang et al. 2001). Second, activation of D_2 receptors does not alter medium spiny neuron activity by inhibiting adenylyl cyclase (at least not detectably); rather, D_2 receptors appear to couple to phospholipase Cβ isoforms to generate diacylglycerol (DAG) and inositol trisphosphate (IP3). This leads to mobilization of intracellular Ca^{2+} stores and activation of PKC. The mobilization of intracellular Ca^{2+} leads to negative modulation of Cav1.3a channels located at excitatory synaptic sites through a calcineurin-dependent mechanism. Activation of PKC enhances the slow inactivation of all Na^+ channel alpha subunits (Nav1.1, Nav1.6 in particular).

The modulation of Cav1.3 and Nav1.1/1.6 Na^+ channels induces an activity-dependent suppression of medium spiny neuron UP state transitions and activity driven by excitatory synaptic input in the slice preparation (Figure 6.4b). The use dependence of the Na^+ channel modulation may strengthen the suppression of UP states as they progress, leading to a shortening in their duration. This feature of the modulation could also serve to diminish the invasion of back-propagating spikes into the dendritic tree, lessening the chances for synaptic strengthening. It is curious, however, that synaptically driven Ca^{2+} flux through L-type Ca^{2+} channels (Cav1.3?) appears to be necessary for long-term synaptic depression in medium spiny neurons (Bonsi et al. 2003).

DOPAMINERGIC MODULATION OF THE INTRINSIC PROPERTIES OF CHOLINERGIC INTERNEURONS

D_2 and D_5 Receptor Activation Leads to a Suppression of Interneuronal Excitability

Striatal cholinergic interneurons are autonomous pacemakers; that is, they spike rhythmically in the absence of synaptic input (Bennett et al. 2000). This intrinsically generated activity relies upon the subthreshold, depolarizing influence provided by voltage-dependent Na^+ channel currents. Activation of interneuronal D_2 receptors reduced both transient and persistent somatic Na^+ currents. The suppression does not depend upon an alteration in the voltage dependence of channel opening or fast inactivation; rather, the reduction in Na^+ channel availability is brought about by an enhanced entry into a slow inactivated state. This endowed the modulation with a profound voltage dependence, being clearly evident at membrane potentials found during pacemaking but virtually absent at more hyperpolarized membrane potentials, like those achieved during rhythmic bursting (Bennett and Wilson 1999).

The D_2 receptor modulation is accomplished through a G$\beta\gamma$-signaling pathway that activates PKC, as in medium spiny neurons. This linkage is consistent with the recent demonstration that phosphorylation of Na^+ channels enhances entry into a slow inactivated state (Carr et al. 2003). In cortical pyramidal

neurons, activation of 5-HT_2 receptors and PKC also suppresses Na^+ currents by promoting entry into a slow inactivated state, as seen following D_2 receptor activation.

The modulation of Na^+ currents should work in concert with D_5 receptor-mediated augmentation of spike afterhyperpolarization to slow discharge in response to elevations in striatal dopamine (Bennett and Wilson 1998), accounting, in part, for conjectured involvement of D_1-class receptors in generation of the pause (Watanabe and Kimura 1998). These alterations in intrinsic properties governing autonomous spiking complement the direct D_2 receptor-mediated inhibition of acetylcholine release (Lehmann and Langer 1983; Bertorelli et al. 1992; Stoof et al. 1992; DeBoer et al. 1993; Di Chiara et al. 1994; Yan et al. 1997), further reducing the impact of interneurons on striatal circuitry.

Dopaminergic regulation of synaptic inputs to cholinergic interneurons may also contribute to the generation of alterations in activity during associative conditioning. Thalamic inputs carrying sensory information are important participants in the regulation of interneuronal activity (Matsumoto et al. 2001). Although there are direct thalamic projections to interneurons (Wilson et al. 1990; Lapper and Bolam 1992), the most important synaptic linkage in this context may be through intrastriatal GABAergic interneurons. Thalamic activation of these neurons following presentation of a sensory cue could act to suppress cholinergic interneuron discharge. However, because thalamic activity is not contingent upon reward, this linkage would have to be gated by dopamine. This could be accomplished by dopaminergic enhancement of GABAergic interneuron activity (Bracci et al. 2002) and of cholinergic interneuron sensitivity to this input (Yan and Surmeier 1997).

In associative learning paradigms, the ongoing activity of primate cholinergic interneurons (or TANs) (Aosaki et al. 1994; Graybiel et al. 1994; Bennett and Wilson 1999) is suppressed by presentation of unconditioned, positive stimuli and by learned, conditioned stimuli. In both cases, the interneuronal suppression is dependent upon stimulus-linked elevations in dopamine cell activity and dopamine release in the striatum (Aosaki et al. 1994).

Work *in vivo* has shown that the pause in interneuronal discharge is primarily mediated by D_2 receptors (Watanabe and Kimura 1998). The experiments reported by Watanabe and Kimura provide a direct linkage between D_2 receptor activation and suppression of Na^+ channel currents known to underlie autonomous spike activity in these neurons. The failure of MPTP lesioning (Aosaki et al. 1994) or D_2 receptor antagonists (Watanabe and Kimura 1998) to suppress spontaneous discharge rates of nominal cholinergic interneurons (TANs) suggests that resting, interneuronal D_2 receptor tone is low and that the augmented dopamine accumulation and volumetric transmission associated with high-frequency discharge following presentation of unconditioned or conditioned reinforcers is necessary for D_2 receptor activation and suppression of Na^+ currents (Chergui et al. 1994; Schultz 2002).

CHOLINERGIC MODULATION OF THE INTRINSIC PROPERTIES OF MEDIUM SPINY NEURONS

M_1 and M_4 Receptor Activation Increases Medium Spiny Neurons Excitability

Both striatopallidal and striatonigral neurons express M_1 and M_4 receptors (Hersch et al. 1994; Yan et al. 2001). M_1 receptors are expressed at uniformly high levels whereas M_4 receptor mRNA is somewhat higher in striatonigral neurons. M_1 receptors are G_q and PLCβ linked. PLC activation results in the metabolism of PIP2 and the production of IP3 and DAG. The depletion of membrane PIP2 leads to a decrement in the open probability of KCNQ and Kir2 channels in medium spiny neurons. Coincident channel phosphorylation by DAG-stimulated PKC amplifies the suppression of KCNQ channel opening. The suppression of Kir2 channels may be stronger in striatonigral than striatopallidal neurons as Kir2 channels in these neurons rely upon Kir2.3 channels, which have a lower affinity for PIP2. Suppression of KCNQ channels increases somatic excitability at depolarized membrane potentials, near the UP state. Suppression of Kir2 channels should increase dendritic input resistance, shorten electrotonic lengths, and increase the somatic impact of glutamatergic synaptic events. In parallel with these excitatory effects, the activation of M_4 receptors suppresses Cav2.2/2.3 Ca^{2+} channel opening via a G$\beta\gamma$ mechanism. These channels have been linked to activation of SK channels (see above). Diminution of SK currents should increase neuronal excitability. In corticostriatal slices, bath application of muscarine increases the occurrence of UP state transitions following cortical stimulation (unpublished observations) in agreement with this pattern of channel modulation.

Some of the other effects of M_1 receptor activation are not as easily reconciled with this model. In particular, M_1 receptor activation suppresses Cav1.3 channel currents, as does D_2 receptor activation. How can these apparently divergent modulations be reconciled? One possibility is that the contrary modulations are gated. This is certainly true for the M_1 modulation of Cav1.3 channels. If D_1 receptors are activated before or during M_1 receptor stimulation, the reduction in Cav1.3 channel currents will be blocked by RCS. This would be an excellent mechanism for detecting the coincidence of cholinergic, dopaminergic, and glutamatergic inputs to a particular neuron.

STRIOSOME/MATRIX CORRELATIONS?

One of the best-described histochemical organizations of the striatum is the striosomal/matrix organization based in part upon the distribution of cholinesterase staining (Graybiel and Ragsdale 1978; Malach and Graybiel 1986). If we assume that cholinergic interneurons are tonically active, the resting tone in

striosomes should be higher than in the ChE-rich matrix. This should lead to an increased excitability of neurons in the striosomes. An interesting observation that may be correlated with this arrangement is that the UP states of some medium spiny neurons are more depolarized than others, leading some medium spiny neurons to spike during UP states whereas others do not (Wickens and Wilson 1998). This difference could reflect striosome/matrix location. (Although the relative proportions of the two types of medium spiny neurons in an anesthetized animal are not consistent with this conjecture, there may be a very strong sampling bias in this study.)

Cholinergic tone in the striatum may create a "listening mode" in medium spiny neurons. Muscarinic receptor tone increases excitability of medium spiny neurons in several ways. The ubiquitous M_1 receptors suppress Kir2 channel currents, making medium spiny neurons more electrotonically compact; they also suppress KCNQ channel currents, increasing somatic excitability by removing a subthreshold K^+ current. M_4 receptor activation diminishes Cav2.1/2.2 Ca^{2+} currents, leading to a reduction in SK K^+ currents, increasing somatic excitability further (this may be more important in SP medium spiny neurons). All of these alterations should increase the responsiveness of medium spiny neurons to glutamatergic synaptic input. Another prominent modulatory target of M_1 receptors is the Cav1.3 L-type Ca^{2+} channel, which appears to be largely dendritic, near excitatory synapses. Suppression of this current by M_1 receptors should diminish the propensity of dendritic regions to exhibit plateau potentials or bistability. However, this modulation is potently blocked by D_1 dopamine receptors and RCS, raising the possibility that in SP medium spiny neurons coincident activation of dopamine and muscarinic receptors creates a significant elevation in excitability and the propensity to bistability. This could promote prolonged UP states in this segment of the medium spiny neuron population. In contrast, both D_2 and M_1 receptors suppress Cav1.3 channel opening in ENK medium spiny neurons.

REFERENCES

Albin, R.L., A.B. Young, and J.B. Penney. 1989. The functional anatomy of basal ganglia disorders. *TINS* **12**:366–375.

Aosaki, T., A.M. Graybiel, and M. Kimura. 1994. Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. *Science* **265**:412–415.

Bading, H., D.D. Ginty, and M.E. Greenberg. 1993. Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. *Science* **260**:181–186.

Bazhenov, M., I. Timofeev, M. Stériade, and T.J. Sejnowski. 2002. Model of thalamocortical slow-wave sleep oscillations and transitions to activated states. *J. Neurosci.* **22**:8691–8704.

Beiser, D.G., and J.C. Houk. 1998. Model of cortical-basal ganglionic processing: Encoding the serial order of sensory events. *J. Neurophysiol*. **79**:3168–3188.

Bennett, B.D., J.C. Callaway, and C.J. Wilson. 2000. Intrinsic membrane properties underlying spontaneous tonic firing in neostriatal cholinergic interneurons. *J. Neurosci*. **20**:8493–8503.

Bennett, B.D., and C.J. Wilson. 1998. Synaptic regulation of action potential timing in neostriatal cholinergic interneurons. *J. Neurosci*. **18**:8539–8549.

Bennett, B.D., and C.J. Wilson. 1999. Spontaneous activity of neostriatal cholinergic interneurons *in vitro. J. Neurosci*. **19**:5586–5596.

Bertorelli, R., M. Zambelli, G. Di Chiara, and S. Consolo. 1992. Dopamine depletion preferentially impairs D_1- over D_2-receptor regulation of striatal *in vivo* acetylcholine release. *J. Neurochem*. **59**:353–357.

Bolam, J.P., J.J. Hanley, P.A. Booth, and M.D. Bevan. 2000. Synaptic organisation of the basal ganglia. *J. Anat*. **196**(Pt 4):527–542.

Bonsi, P., A. Pisani, G. Bernardi, and P. Calabresi. 2003. Stimulus frequency, calcium levels, and striatal synaptic plasticity. *Neuroreport* **14**:419–422.

Bracci, E., D. Centonze, G. Bernardi, and P. Calabresi. 2002. Dopamine excites fast-spiking interneurons in the striatum. *J. Neurophysiol*. **87**:2190–2194.

Carr, D.B., M. Day, A.R. Cantrell et al. 2003. Transmitter modulation of slow, activity-dependent alterations in sodium channel availability endows neurons with a novel form of cellular plasticity. *Neuron* **39**:793–806.

Cepeda, C., N.A. Buchwald, and M.S. Levine. 1993. Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. *PNAS* **90**:9576–9580.

Cepeda, C., C.S. Colwell, J.N. Itri, S.H. Chandler, and M.S. Levine. 1998. Dopaminergic modulation of NMDA-induced whole-cell currents in neostriatal neurons in slices: Contribution of calcium conductances. *J. Neurophysiol*. **79**:82–94.

Chergui, K., M.F. Suaud-Chagny, and F. Gonon. 1994. Nonlinear relationship between impulse flow, dopamine release, and dopamine elimination in the rat brain *in vivo*. *Neuroscience* **62**:641–645.

DeBoer, P., E.D. Abercrombie, M. Heeringa, and B.H. Westerink. 1993. Differential effect of systemic administration of bromocriptine and L-dopa on the release of acetylcholine from striatum of intact and 6-OHDA-treated rats. *Brain Res*. **608**:198–203.

Di Chiara, G., M. Morelli, and S. Consolo. 1994. Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. *TINS* **17**:228–233.

Flores-Hernandez, J., C. Cepeda, E. Hernandez-Echeagaray et al. 2002. Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: Role of D1 receptors and DARPP-32. *J. Neurophysiol*. **88**:3010–3020.

Gerfen C.R. 1992. The neostriatal mosaic: Multiple levels of compartmental organization. *TINS* **15**:133–139.

Gong, B., K.J. Rhodes, Z. Bekele-Arcuri, and J.S. Trimmer. 1999. Type I and type II $Na(^{+})$ channel alpha-subunit polypeptides exhibit distinct spatial and temporal patterning, and association with auxiliary subunits in rat brain. *J. Comp. Neurol*. **412**:342–352.

Graybiel, A.M. 1986. Neuropeptides in the basal ganglia. *Res. Publ. Assoc. Res. Nerv. Ment. Dis*. **64**:135–161.

Graybiel, A.M., T. Aosaki, A.W. Flaherty, and M. Kimura. 1994. The basal ganglia and adaptive motor control. *Science* **265**:1826–1831.

Graybiel, A.M., and C.W. Ragsdale, Jr. 1978. Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. *PNAS* **75**:5723–5726.

Hersch, S.M., C.A. Gutekunst, H.D. Rees, C.J. Heilman, and A.I. Levey. 1994. Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: Light and electron microscopic immunocytochemistry using subtype-specific antibodies. *J. Neurosci*. **14**:3351–3363.

Jiang, M., K. Spicher, G. Boulay, Y. Wang, and L. Birnbaumer. 2001. Most central nervous system D2 dopamine receptors are coupled to their effectors by Go. *PNAS* **98**:3577–3582.

Kawaguchi, Y., C.J. Wilson, S.J. Augood, and P.C. Emson. 1995. Striatal interneurones: Chemical, physiological, and morphological characterization. *TINS* **18**:527–535.

Kerr, J.N., and D. Plenz. 2002. Dendritic calcium encodes striatal neuron output during UP states. *J. Neurosci.* **22**:1499–1512.

Konradi, C., J.C. Leveque, and S.E. Hyman. 1996. Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. *J. Neurosci.* **16**:4231–4239.

Lapper, S.R., and J.P. Bolam. 1992. Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. *Neuroscience* **51**:533–545.

Lehmann, J., and S.Z. Langer. 1983. The striatal cholinergic interneuron: Synaptic target of dopaminergic terminals? *Neuroscience* **10**:1105–1120.

Levine, M.S., K.L. Altemus, C. Cepeda et al. 1996. Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D1A-deficient mutant mice. *J. Neurosci*. **16**:5870–5882.

Malach, R., and A.M. Graybiel. 1986. Mosaic architecture of the somatic sensory-recipient sector of the cat's striatum. *J. Neurosci*. **6**:3436–3458.

Matsumoto, N., T. Minamimoto, A.M. Graybiel, and M. Kimura. 2001. Neurons in the thalamic CM-Pf complex supply striatal neurons with information about behaviorally significant sensory events. *J. Neurophysiol*. **85**:960–976.

Maurice, N., J. Mercer, C.S. Chan et al. 2004. D_2 dopamine receptor-mediated modulation of voltage-dependent Na^+ channels reduces autonomous activity in striatal cholinergic interneurons. *J. Neurosci.* **24**:10,289–10,301.

Mermelstein, P.G., W.J. Song, T. Tkatch, Z. Yan, and D.J. Surmeier. 1998. Inwardly rectifying potassium (IRK) currents are correlated with IRK subunit expression in rat nucleus accumbens medium spiny neurons. *J. Neurosci*. **18**:6650–6661.

Nicola, S.M., and R.C. Malenka. 1998. Modulation of synaptic transmission by dopamine and norepinephrine in ventral but not dorsal striatum. *J. Neurophysiol*. **79**: 1768–1776.

Nisenbaum, E.S., and C.J. Wilson. 1995. Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. *J. Neurosci*. **15**: 4449–4463.

Nisenbaum, E.S., Z.C. Xu, and C.J. Wilson. 1994. Contribution of a slowly inactivating potassium current to the transition to firing of neostriatal spiny projection neurons. *J. Neurophysiol*. **71**:1174–1189.

Olson, P.A., T. Tkatch, S. Hernandez-Lopez et al. 2005. G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca^{2+} channels is dependent on a Shank-binding domain. *J. Neurosci.* **25**:1050–1062.

Rakhilin, S.V., P.A. Olson, A. Nishi et al. 2004. A network of control mediated by regulator of calcium/calmodulin-dependent signaling (RCS). *Science* **306**:698–701.

Raz, A., V. Frechter-Mazar, A. Feingold et al. 2001. Activity of pallidal and striatal tonically active neurons is correlated in mptp-treated monkeys but not in normal monkeys. *J. Neurosci*. **21**:RC128.

Schultz, W. 2002. Getting formal with dopamine and reward. *Neuron* **36**:241–263.

Shen, W., S. Hernandez-Lopez, T. Tkatch, J.E. Held, and D.J. Surmeier. 2004. Kv1.2-containing K^+ channels regulate subthreshold excitability of striatal medium spiny neurons. *J. Neurophysiol*. **91**:1337–1349.

Sheng, M., and E. Kim. 2000. The Shank family of scaffold proteins. *J. Cell Sci*. **113**(Pt 11):1851–1856.

Snyder, G.L., A.A. Fienberg, R.L. Huganir, and P. Greengard. 1998. A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. *J. Neurosci*. **18**:10297–10303.

Soom, M., R. Schonherr, Y. Kubo et al. 2001. Multiple PIP2 binding sites in Kir2.1 inwardly rectifying potassium channels. *FEBS Lett*. **490**:49–53.

Stoof, J.C., B. Drukarch, P. de Boer, B.H. Westerink, and H.J. Groenewegen. 1992. Regulation of the activity of striatal cholinergic neurons by dopamine. *Neuroscience* **47**:755–770.

Surmeier, D.J., J. Bargas, H.C. Hemmings, Jr., A.C. Nairn, and P. Greengard. 1995. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* **14**:385–397.

Surmeier, D.J., W.J. Song, and Z. Yan. 1996. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci*. **16**:6579–6591.

Tkatch, T., G Baranauskas., and D.J. Surmeier. 2000. Kv4.2 mRNA abundance and A-type $K(^+)$ current amplitude are linearly related in basal ganglia and basal forebrain neurons. *J. Neurosci*. **20**:579–588.

Townsend, C., and R. Horn. 1997. Effect of alkali metal cations on slow inactivation of cardiac Na^+ channels. *J. Gen. Physiol*. **110**:23–33.

Uchimura, N., E. Cherubini, and R.A. North. 1989. Inward rectification in rat nucleus accumbens neurons. *J. Neurophysiol*. **62**:1280–1286.

Vergara, R., C. Rick, S. Hernandez-Lopez et al. 2003. Spontaneous voltage oscillations in striatal projection neurons in a rat corticostriatal slice. *J. Physiol*. **553**:169–182.

Vilchis, C., J. Bargas, G.X. Ayala, E. Galvan, and E. Galarraga. 2000. Ca^{2+} channels that activate Ca^{2+}-dependent K^+ currents in neostriatal neurons. *Neuroscience* **95**:745–752.

Watanabe, K., and M. Kimura. 1998. Dopamine receptor-mediated mechanisms involved in the expression of learned activity of primate striatal neurons. *J. Neurophysiol*.**79**:2568–2580.

Wickens, J.R., and C.J. Wilson. 1998. Regulation of action-potential firing in spiny neurons of the rat neostriatum *in vivo*. *J. Neurophysiol*. **79**:2358–2364.

Wilson, C.J. 1993. The generation of natural firing patterns in neostriatal neurons. *Prog. Brain Res*. **99**:277–297.

Wilson, C.J., H.T. Chang, and S.T. Kitai. 1990. Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. *J. Neurosci*. **10**:508–519.

Wilson, C.J., and Y. Kawaguchi. 1996. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J. Neurosci*. **16**:2397–2410.

Xiao, J., X.G. Zhen, and J. Yang. 2003. Localization of PIP2 activation gate in inward rectifier K^+ channels. *Nat. Neurosci*. **6**:811–818.

Yan, Z., J. Flores-Hernandez, and D.J. Surmeier. 2001. Coordinated expression of muscarinic receptor messenger RNAs in striatal medium spiny neurons. *Neuroscience* **103**:1017–1024.

Yan, Z., W.J. Song, and D.J. Surmeier. 1997. D_2 dopamine receptors reduce N-type Ca^{2+} currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. *J. Neurophysiol*. **77**:1003–1015.

Yan, Z., and D.J. Surmeier. 1997. D5 dopamine receptors enhance Zn^{2+}-sensitive GABA(A) currents in striatal cholinergic interneurons through a PKA/PP1 cascade. *Neuron* **19**:1115–1126.

7

Microcircuits in the Striatum

Striatal Cell Types and Their Interaction

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ABSTRACT

The neostriatum is strategically located in the forebrain and receives inputs from all cortical areas. The complexity of the corticostriatal pathways suggests that striatal neurons are in a unique position to process convergent inputs from cortex and through basal ganglia output nuclei to control subcortical nuclei and/or contribute to cortical dynamics via the thalamus. The most abundant neuron in the striatum, the GABAergic spiny projection neuron, has been at the center of research on how cortical inputs are evaluated at the striatal level. Because of the spiny neuron's numerous axon collaterals, the striatum has been viewed historically as a lateral inhibitory network, where cortical inputs compete at the striatal level for control of basal ganglia output. However, electrophysiological evidence for the existence of the recurrent feedback inhibition between spiny projection neurons has only been forthcoming during the last two years and does not fit the relatively simple, previously suggested role of a selection circuit. Similarly, striatal interneurons (e.g., the fast-spiking interneuron), despite their small numbers, have been recognized increasingly to play a dominant role for certain microcircuit aspects, such as feedforward inhibition and neuromodulation. Finally, the striatum, which receives the densest dopaminergic input in the brain, reveals a highly heterogeneous dopamine receptor composition, suggesting that the striatal microcircuitry is modulated by these dopaminergic inputs in several different ways. This chapter reviews the basic neurocytology, neurophysiology, and connections of the components of the neostriatum and how they interact to generate the neostriatal microcircuitry.

INTRODUCTION

The mammalian neostriatum is the largest nucleus of the basal ganglia and comprises its major input structure. Although anatomical and physiological studies of the basal ganglia and its afferent and efferent connections date back hundreds

of years, it is only within the last quarter century that significant progress has been made to identify clearly the different neuronal types making up the striatum and their electrophysiological properties. The vast majority of striatal neurons are the medium spiny GABAergic neurons, which have been estimated to comprise between 80% and 97.7% of all striatal neurons, depending on species and counting method (Graveland and Difiglia 1985; Gerfen and Wilson 1996; Rymar et al. 2004). These neurons have extensive local axon collaterals that intercalate with and extend beyond the boundary of the dendritic field of the parent neuron. Electron microscopic examination of intracellularly labeled spiny neurons revealed that the local axonal collaterals of these cells form synapses principally with other nearby medium spiny neurons (for a review, see Gerfen and Wilson 1996). Thus it was expected that a major principle of striatal organization would be lateral inhibition among the spiny projection neurons, and many or most models of striatal function have included various instantiations of lateral inhibition as a major element (e.g., Groves 1983; Wickens et al. 1991). However, despite many attempts (e.g., Jaeger et al. 1994), direct electrophysiological evidence supporting lateral inhibition among spiny neurons was not forthcoming, until very recently.

A clearer understanding of the functional internal microcircuitry of the striatum started to emerge during the last decades of the twentieth century, beginning with the discovery of immunocytochemical markers for different striatal interneurons. With the various interneuron types present in relative abundances of 1–2% or less, it was very difficult to obtain recordings from them, *in vivo* or *in vitro*, particularly in large enough numbers to be able to make conclusive statements about the correlation between electrophysiological properties, morphology, and/or neurochemistry. However, taking advantage of infrared illumination and differential interference contrast optics has allowed visually guided whole-cell recordings of striatal neurons *in vitro* and paired recordings between different types of striatal interneurons and projection to be performed with relative ease in striatal brain slices and organotypic cultures. These technical advances have paved the way for the analysis of the striatal microcircuitry, which is the focus of this chapter.

THE NEURON TYPES

Although the existence of several different cell types in striatum, based on Golgi studies, has been acknowledged at least as far back as the work of Cajal (e.g., Groves 1983), the current view of the classification of striatal cell types is based largely on correlation between electrophysiological properties, mainly recorded *in vitro* with intracellular or whole-cell recordings, and single-cell labeling and immunocytochemistry. Since space is limited and the main thrust of this chapter is to discuss striatal microcircuitry, we refer the reader to any of a number of excellent papers and reviews on the neurocytology, neurophysiology, and

connectivity of striatal neurons (e.g., Groves 1983; Kawaguchi 1993; Bolam and Bennett 1995; Kawaguchi et al. 1995; Gerfen and Wilson 1996; Wilson 2004). In this section, we briefly summarize the most salient aspects of this information with regard to striatal microcircuits.

Spiny Projection Neuron

The spiny projection neuron is GABAergic, although it stains significantly less intensely for GABA and the GABA synthetic enzyme, GAD67, than some of the striatal GABAergic interneurons (Kita and Kitai 1988). The soma is medium-sized (12–20 μm) and emits 5–10 smooth primary dendrites that start to become densely invested with spines about 20 μm from the soma (Gerfen and Wilson 1996).

The dendrites branch moderately, yielding a total of 30–35 dendritic tips, and form a roughly spherical arbor 200–300 μm in diameter (Tepper et al. 1998) unless distorted by the presence of a boundary between the striosome and matrix compartments, which they do not cross (Wilson 2004). Spiny projection neurons comprise two major subpopulations distinguished by their principal terminal fields and expression of neuropeptides and transmitter receptors. A full discussion of these characteristics is beyond the scope of this chapter, but in brief, about half of the spiny projection neurons express substance P and dynorphin, and D_1 dopamine receptors, and project predominantly to the substantia nigra and the entopeduncular nucleus (rodent homologue of the internal segment of the globus pallidus), whereas the other half express enkephalin, D_2 dopamine receptors, and project predominantly to the globus pallidus (Bolam and Bennett 1995; Gerfen and Wilson 1996; Wilson 2004). These two cell types are not distinguishable morphologically or electrophysiologically.

Spiny projection neurons possess an extensive local axon collateral system that usually extends roughly through and a bit beyond the volume of the dendritic arborization (e.g., Figure 7.1), although some neurons have a more extensive axon arborization occupying many times the volume of the parent dendritic field. The major postsynaptic targets of these axon collaterals are other spiny neurons, where most of the synapses are formed onto the dendrites or spine necks, and a smaller proportion onto the soma (Gerfen and Wilson 1996). Some of the local collaterals also form symmetric synapses onto the cholinergic interneurons, which also synapse onto spiny projection neurons.

The major excitatory inputs to the spiny neurons come from virtually all areas of the cerebral cortex and from the midline and intralaminar thalamic nuclei. Of these glutamatergic afferents, the vast majority of the corticostriatal inputs ($\sim$90%) as well as those arising from the centromedian and paracentral nuclei are made onto the heads of dendritic spines, whereas those from the parafascicular nucleus predominantly target dendritic shafts (Bolam and Bennett 1995).

Spiny neurons also receive inputs from the cholinergic and GABAergic interneurons (see below). Interestingly, although some of the inputs from the

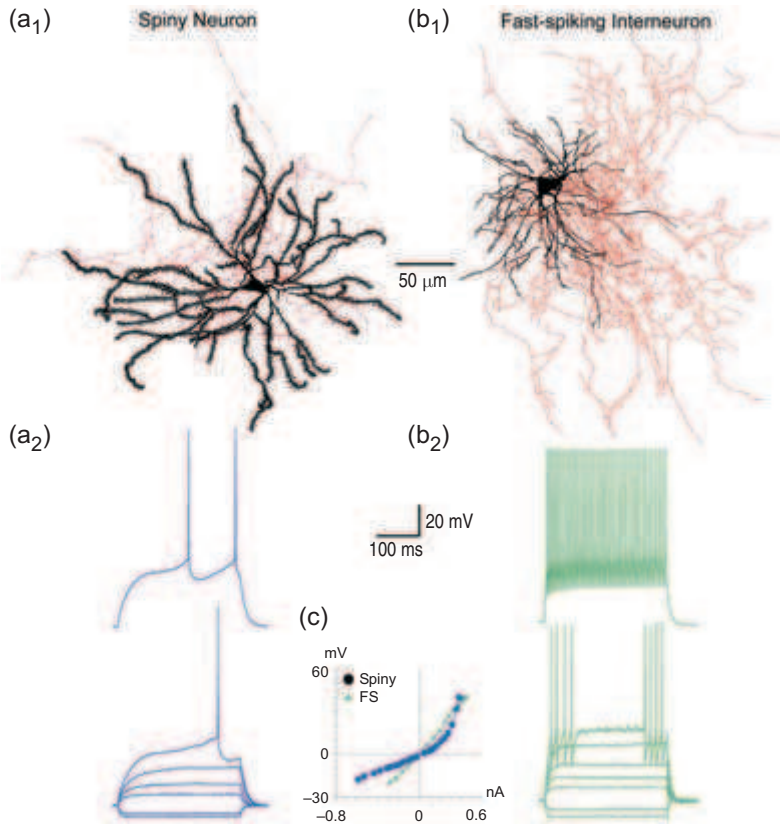

Figure 7.1 Basic anatomical and electrophysiological characteristics of spiny projection neurons and fast-spiking (FS) interneurons. (a_1) Drawing tube reconstruction of a spiny projection neuron stained with biocytin following intracellular recording *in vivo*. Soma and dendrites in black; axon in red. (a_2) Whole-cell current clamp recordings of an adult spiny projection neuron *in vitro* showing the characteristic marked inward rectification and long latency to first spike following suprathreshold-depolarizing current injections. (b_1) Drawing tube reconstruction of an FS interneuron stained with biocytin following whole-cell recording in acute brain slice from a 21-day-old animal. Soma and dendrites in black; axon in red. Note the short dendrites and extremely dense local axonal field. (b_2) Whole-cell current clamp recordings of a 21-day-old FS interneuron *in vitro* showing the characteristic episodic burst firing in response to just suprathreshold depolarizing current injections and the high-frequency firing in response to larger currents. (c) Complete IV plots of the neurons are shown in a_2 and b_2. Note that the interneuron's curve is relatively linear over a large voltage range, whereas the spiny neuron's IV relation shows the marked curvature of inward rectification.

parvalbumin-positive interneurons form symmetric synapses out on the dendrites, the majority of the parvalbumin-immunopositive boutons appear to form pericellular baskets around the cell bodies of the spiny neurons (Bennett and Bolam 1994).

Spiny neurons are the principal target of the dopaminergic afferents from the midbrain, most of which arise from the substantia nigra pars compacta, although some of the dopaminergic input also comes from the ventral tegmental area and the retrorubral field. There is also a sizeable serotonergic input arising mostly from the dorsal raphe nucleus (Wilson 2004).

Spiny projection neurons exhibit a very characteristic pattern of spontaneous activity. The membrane potential of the spiny projection neuron *in vivo* fluctuates between a relatively hyperpolarized "DOWN" state near –80 mV and a relatively depolarized "UP" state near –50 mV (e.g., Figure 7.2). The neurons never fire from the DOWN state, which is characterized by the relative absence of inputs, and may or may not fire during the UP state, creating an extremely phasic or episodic bursty pattern of activity with a low mean firing rate. The UP and DOWN states are known to result from synchronous phasic inputs from large numbers of cortical and/or thalamic neurons that interact with a strong, fast inward rectifier and an outward rectifier (Gerfen and Wilson 1996; Wilson 2004) and possibly GABAergic inputs as well (Plenz 2003). As one would predict from this, the UP state is missing in the absence of synchronous excitatory synaptic inputs such as in the acute slice preparation where these neurons exhibit a resting membrane potential near –80 mV, approximately at the DOWN state membrane potential. On the other hand, striatal UP and DOWN state transitions are readily established in cortex–striatum–substantia nigra organotypic slice cultures in which spontaneous activity in the cortical network provides input to the striatal culture (Plenz and Kitai 1998), as shown in Figure 7.2.

Large Aspiny Cholinergic Interneuron

Large aspiny interneurons were identified in the earliest Golgi studies of the striatum along with the spiny neurons (and were in fact presumed to be the projection neurons until retrograde tracing studies conclusively identified the spiny neurons as the projection neurons in the 1970s). There are, in fact, two different classes of interneurons with large cell bodies (up to 60 μm in diameter) in the striatum. One of them, the large aspiny interneuron, is immunopositive for choline acetyltransferase. The other is the large-sized variant of the fast-spiking (FS) interneuron that expresses parvalbumin (see below).

The cholinergic interneuron is the most abundant of the striatal interneurons comprising 0.3–2% of the neurons in the rodent striatum (Kawaguchi et al. 1995; Rymar et al. 2004). The somata of these interneurons range from 20–50 μm in diameter. The neurons emit 2–4 large primary dendrites, which give rise to higher-order dendrites that span an area up to a millimeter in diameter. Although clearly an aspiny neuron, the distal dendrites may be sparsely invested with spine-like appendages. Unlike the spiny projection neurons, the cholinergic interneurons do not respect the striosome/matrix boundaries and are found in both compartments. Their axons, however, appear to be largely restricted to the matrix (Gerfen and Wilson 1996).

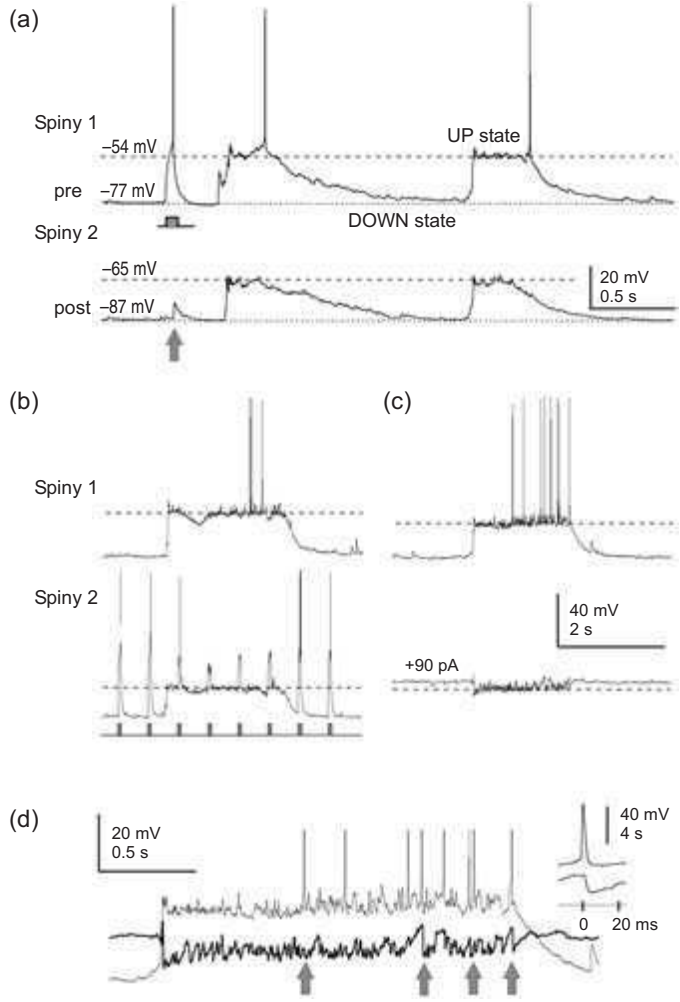

Figure 7.2 Paired whole-cell recordings of spiny neurons in organotypic co-cultures. (a) Spontaneous cortical activity in the culture (not shown) drives spiny neurons through UP and DOWN state transitions. UP states are characterized by a relatively fast transition from the DOWN state at ∼ –80 mV to the depolarized UP state during which the membrane potential remains within a relatively narrow range. The return to the DOWN state is slower. Despite the large depolarization of the UP state, spiny neurons often do not fire action potentials at all or action potential firing is sparse and irregular. (b) UP states are driven by synaptic inputs as can be demonstrated by the reduction in apparent input resistance (spiny neuron 2, SP2) and (c) the reversal of UP states at the chloride reversal potential (E_{Cl-} set to –60 mV). (d) Enlarged time course of membrane trajectories in (c). Inset shows spike-triggered average of the UP state membrane potential in SP2 in response to the action potentials fired during the UP state in SP1. Note that a spontaneous spike in SP1 is followed by a hyperpolarization in SP2. The connection from SP1 → SP2 was demonstrated at rest in response to a brief somatic current injection; see arrow in (a).

The axon of the cholinergic interneuron arises from a proximal dendrite and branches repeatedly to form a dense arborization. The axonal field is usually larger than that of the dendrites of the parent neuron, extends over a large region of the striatum, and may be eccentrically placed relative to the dendritic field (Gerfen and Wilson 1996; Wilson 2004). The cholinergic neuron synapses onto the medium spiny neuron and also innervates FS interneurons as well (Koós and Tepper 2002).

The predominant excitatory input to the cholinergic interneuron arises from the thalamus (Bolam and Bennett 1995). There may also be a cortical input that is restricted to the distal dendrites. There is also a monosynaptic input from the dopaminergic neurons of the substantia nigra pars compacta.

Cholinergic interneurons exhibit broad action potentials and prominent slow spike afterhyperpolarizations due to the presence of a high threshold voltage-activated calcium conductance and a calcium-activated potassium conductance. The neurons also exhibit a prominent hyperpolarization-activated current as well as a persistent Na conductance. These conductances interact to allow the cholinergic interneurons to fire spontaneously in the absence of any inputs, most often in a rhythmic pacemaker mode. However, the neurons can also fire in bursts or in an irregular pattern, both *in vivo* or *in vitro* (Bennett and Wilson 1999; Wilson 2004). Compared to the spiny projection neurons, the cholinergic interneuron exhibits a relatively depolarized resting membrane potential between –55 and –60 mV, only a few millivolts below spike threshold, and does not display the discrete UP and DOWN states of the spiny neuron.

GABAergic Interneurons

There are now known to be at least three distinct classes of striatal GABAergic interneurons, distinguishable on electrophysiological, morphological, and/or neurochemical bases.

Parvalbumin-containing GABAergic Interneurons

A population of medium-sized striatal GABAergic interneurons was originally described on the basis of selective accumulation of radiolabeled GABA (Bolam and Bennett 1995). Subsequent studies also revealed that these neurons were among a population of neurons that expressed immunoreactivity for GAD and for GABA much more intensely than the medium spiny neurons (Kita and Kitai 1988). It was subsequently shown that many of these intensely GABAergic interneurons are also immunopositive for the calcium-binding protein, parvalbumin. Recent stereological estimates of the proportion of parvalbumin-positive neurons puts them at slightly less than 1% (Rymar et al. 2004). There is a strong mediolateral gradient in the distribution of parvalbumin-positive axons and terminals, which suggests that these cells may be more integral to functioning in lateral striatum than medial striatum (Bolam and Bennett 1995).

There is some morphological heterogeneity among parvalbumin-positive interneurons. They range in size from medium-sized neurons that are about the size of the spiny projection neurons or perhaps slightly larger, to large neurons with somata as large as those of the cholinergic interneurons. The smaller variety exhibits relatively short (50–100 μm) aspiny dendrites that are sparsely branched and generally smooth, but which in some cells are highly varicose. The larger of the cells may exhibit a dendritic field about double the diameter of the smaller (Kawaguchi 1993; Kawaguchi et al. 1995; Koós and Tepper 1999). The local axonal plexus is exceedingly dense and in most cases is co-extensive although slightly larger than the dendritic arborization of the parent neuron, as shown in Figure 7.1. Parvalbumin-positive interneurons primarily target the cell bodies and proximal dendrites of medium spiny neurons, and individual parvalbumin-positive axons form pericellular baskets around spiny neuron somata (Bennett and Bolam 1994).

Parvalbumin-positive interneurons receive a powerful excitatory input from the neocortex as well as input from the local axon collaterals of medium spiny neurons. An additional extrinsic inhibitory input arises from a subpopulation of GABAergic globus pallidus projection neurons, and the cholinergic interneuron provides a second intrinsic input (Bolam and Bennett 1995; Kawaguchi et al. 1995; Koós and Tepper 1999).

The parvalbumin-positive interneuron exhibits a characteristic electrophysiological phenotype shared by GABAergic parvalbumin-positive interneurons in other brain regions including the neocortex and the hippocampus where they are referred to as basket cells or, more recently, FS interneurons. The FS interneurons exhibit very narrow action potentials, relatively linear IV relations, and can fire sustained trains of action potentials at rates up to 300 Hz with little evidence of spike frequency adaptation, as shown in Figure 7.1. Although the I-F functions of these neurons are relatively linear at above 20 Hz, the neurons cannot be made to fire arbitrarily slowly, and peri-threshold depolarizing current injections give rise to aperiodic bursts of action potentials of varying lengths, sometimes preceded by a ramp-like depolarization (Figures 7.1b; Kawaguchi 1993; Kawaguchi et al. 1995).

The spontaneous activity pattern of FS interneurons *in vivo* is not very well documented (Kita 1993). Their vigorous response to supra-threshold somatic current injection suggests that these neurons fire a burst of action potentials when receiving excitatory (e.g., cortical) inputs. However, this type of spontaneous bursting is rarely observed in cortex–striatum–substantia nigra co-cultures where FS neurons are spontaneously active when spiny projection neurons are in the UP state (Figure 7.3g). The most common expression of spontaneous activity in FS interneurons in these organotypic slice cultures is single action potential firing during UP state periods. Only occasionally, particularly during the initial period when spiny projection neurons transition into an UP state, is burst firing observed in FS neurons. During the DOWN state period, FS interneurons

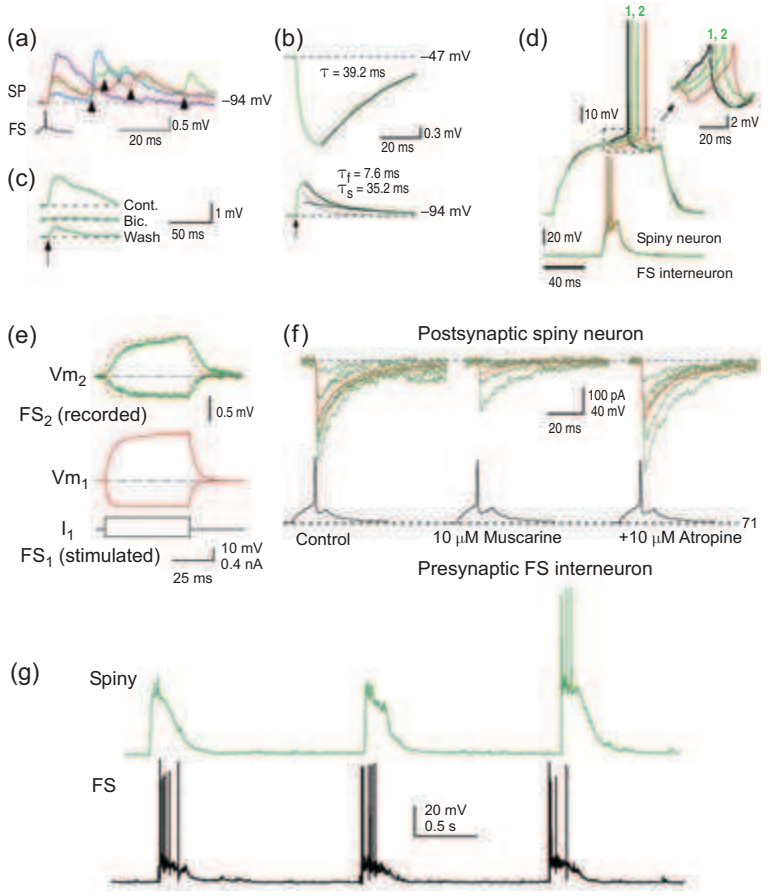

Figure 7.3 Electrophysiological characteristics of the fast-spiking (FS) spiny cell synapse in acute slices from adult rats. (a) Variable amplitude inhibitory postsynaptic potentials (IPSPs) evoked in a spiny neuron at rest by an FS interneuron (presynaptic spikes indicated by the arrow and arrowheads). (b) The amplitude and time course of the IPSP is strongly influenced by the postsynaptic membrane potential. (c) IPSP can be reversibly blocked with 20 μM bicuculline. (d) A single action potential elicited in a spiny neuron by current injection (2 upper green traces) is delayed by IPSPs evoked by single spikes (lower green trace) or a spike doublet (lower red trace) of an FS interneuron. The delay is variable (cf. green spikes 1, 2), and the spike doublet (red traces) is more effective than single spikes. (e) Electrical coupling among FS interneurons. Dashed red line in the upper panel (Vm_2) is the normalized response of FS_1. Note the electrotonic distortion and delay of the response in FS_2. (f) Single action potentials of the FS interneuron elicited IPSCs (green sweeps) with variable amplitudes (left). Bath application of 10 μM muscarine strongly reduced the average amplitude of the postsynaptic response without affecting the FS interneuron. Co-application of 10 μM atropine reversed the effect of muscarine (right). Red traces are population mean. (g) Spontaneous firing of FS interneurons during UP states in spiny neurons (cortex–striatum–substantia nigra culture). (a)–(e) modified with permission from Koós and Tepper (1999); (f) modified with permission from Koós and Tepper (2002).

are ~10–15 mV more depolarized than spiny projection neurons but do not fire action potentials to a very hyperpolarized membrane potential (~ –80 mV).

Another characteristic feature of these interneurons is that they are interconnected by functional electrotonic gap junctions. These have been described at the electron microscopic level (Kita et al. 1990) as well as by demonstration of electrotonic coupling *in vitro*. In slices from adult striatum, the coupling ratio ranges between 3% and 20% (Koós and Tepper 1999).

Somatostatin-containing GABAergic Interneurons

A second population of GABAergic interneurons comprises a medium-sized aspiny neuron that expresses somatostatin, nitric oxide synthase (NOS), NADPH diaphorase, neuropeptide Y (NPY), and GABA. These neurons are estimated to be approximately as abundant as the parvalbumin-containing neurons, in rat just less than 1% of the total (Rymar et al. 2004). The dendrites are more sparsely branched than those of the parvalbumin-containing interneuron and are smooth and aspiny.

The axonal arborization of the somatostatin-containing interneuron is less dense than that of any of the other interneurons and generally extends beyond the volume of the dendritic arborization. The neurons receive excitatory monosynaptic input from neocortex and thalamus and, like parvalbumin-containing interneurons, also receive an input from the globus pallidus (Bolam and Bennett 1995). Both ChAT- and TH-positive terminals form symmetric synapses onto somatostatin-containing neurons (Bolam and Bennett 1995; Kawaguchi et al. 1995).

Somatostatin-containing interneurons *in vitro* exhibit a relatively depolarized resting membrane potential (~ –56 mV) and high input resistance, significantly greater than that of spiny projection neurons or FS interneurons. The most characteristic electrophysiological properties of these neurons are the presence of low-threshold calcium spikes (LTS) and a persistent Ca^{2+}-sensitive depolarization upon cessation of hyperpolarizing pulses; hence these neurons are referred to by some authors as LTS or P(ersistent)LTS neurons (Kawaguchi 1993; Koós and Tepper 1999).

Calretinin-containing GABAergic Interneurons

The third and least well-characterized subtype of GABAergic interneuron is a medium-sized aspiny interneuron that co-localizes the calcium-binding protein, calretinin. A recent stereological study gives the relative abundance of the calretinin interneuron in rats as 0.5%. There is a decreasing gradient of expression from rostral to caudal (Rymar et al. 2004). As there are no reports of single-cell recording or filling of these neurons, very little is known of their anatomy, afferent or efferent projections, or electrophysiological properties, and they will not be considered further here.

INTRASTRIATAL CIRCUITRY

Feedforward Inhibition in Striatum

Although the spiny cell axon collaterals are more numerous and have long been recognized as likely mediators of important intrastriatal circuitry, early experiments pointed towards feedforward inhibition mediated by striatal GABAergic interneurons as the most potent source of intrastriatal inhibition. This was verified directly *in vitro* with paired whole-cell recordings from organotypic co-cultures of basal ganglia and neostriatal slices. These experiments revealed that FS interneurons synapsed monosynaptically on spiny projection neurons where they produced large $GABA_A$-mediated inhibitory postsynaptic potentials (IPSPs) (Plenz and Kitai 1998; Koós and Tepper 1999). Most importantly, the inhibition from a single FS interneuron was strong enough to delay or inhibit action potential firing in a synaptically connected spiny projection neuron (Koós and Tepper 1999). Furthermore, these synapses exhibited a very low failure rate (<1%; Koós and Tepper 1999). The synapse is strong enough to impose the temporal firing pattern from the interneuron on spiny projection neuron firing. Such a function would be supported by synaptic short-term depression found in organotypic cultures (Plenz and Kitai 1998) and which is known to preserve temporal input structure (Figure 7.4b). This strong inhibition of the projection neuron was not limited to FS interneurons, however, as LTS neurons were also shown to potently inhibit spiny projection neurons (Koós and Tepper 1999).

Based on estimates of the frequency of synaptic connectivity among FS interneurons and spiny projection neurons in acute slices (25% for pairs within 250 μm) and the volumes of the axonal and dendritic arborizations of FS interneurons and spiny projection neurons, Koós and Tepper (1999) suggested that a single FS interneuron contacts approximately 135–541 spiny neurons and between 4–27 FS neurons converge onto one spiny neuron. This suggests that the population of FS neurons is in a prime position to provide an efficient feedforward inhibition onto spiny projection neurons. The feedforward inhibition is very different from the feedback GABAergic circuit provided by spiny projection neurons, because in feedforward inhibition, the outcome of the operation (the neuron, which processes the inhibition) does not influence the inhibition itself. This difference and the recent suggestion that the striatal FS interneuron might receive cortical inputs distinct from those to spiny projection neurons, points to this part of the intrastriatal GABAergic circuitry as subserving a different function than the axon collateral system (see below).

This raises the question as to whether firing in FS interneurons reveals some temporal structure. A network of mutually coupled interneurons, when broadly activated, can stabilize into synchronized population firing at beta and gamma frequencies. If present in the striatum, the interneuron network state would force spiny projection neurons to fire only at defined gaps during feedforward inhibition. Whether FS interneurons can support such a role in the neostriatum is still

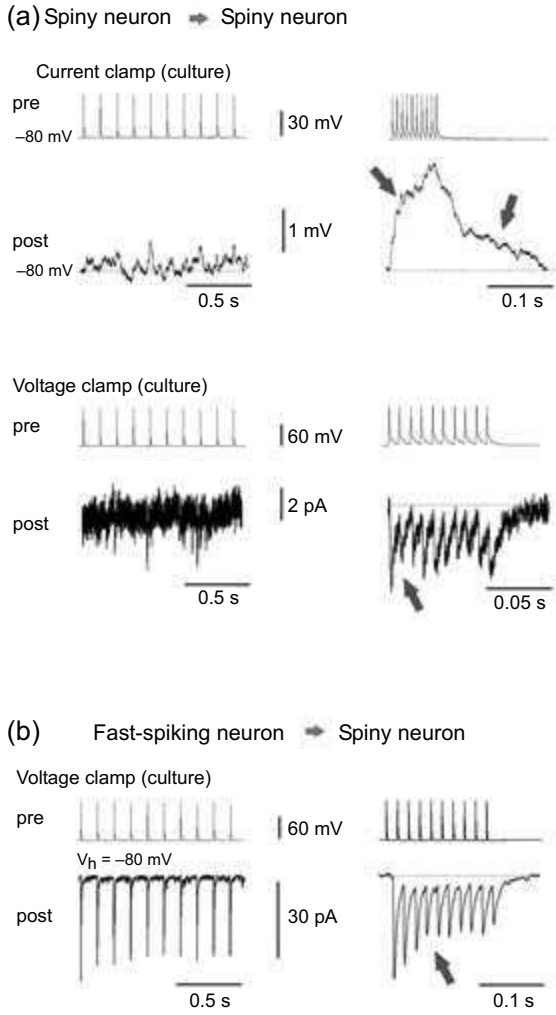

Figure 7.4 Short-term plasticity in synaptic connections from spiny neurons and fast-spiking (FS) interneurons in organotypic co-cultures. (a) High failure rates and short-term facilitation in connections between spiny projection neurons. Note high failure rate of synaptic connection at low presynaptic action potential frequency of 10 Hz. The same synaptic connection and number of action potentials depolarizes the somatic membrane potential by up to 2 mV if the frequency of the presynaptic spike train increases to 80 Hz. Note the initial facilitation and the depolarization that outlasts the presynaptic action potential burst (arrows). The short-term facilitation is also clearly observed in voltage clamp. Note initial short-term depression in response to the first three action potentials. Each response is an average over 5 stimuli. (b) Low failure rate and short-term depression in a connection from an FS interneuron to a spiny neuron. All presynaptic action potentials were precisely timed using exponentially decaying positive current injections. Note the prominent short-term depression that develops after the first action potential.

unclear. In contrast to cortical layer V FS interneurons, striatal FS interneurons have not yet been shown to innervate each other through chemical synapses, despite extensive coupling through electrotonically active gap junctions (Koós and Tepper 1999). Recent findings of oscillatory local field potential activity in the striatum in the awake monkey (Courtemanche et al. 2003), however, hint at the presence of such a mechanism.

Collateral Inhibition between Striatal Spiny Projection Neurons

Historically, a local interaction between spiny projection neurons has always played an important role in models of the striatum and basal ganglia (for a review, see Plenz 2003). The main driving force behind this idea, other than the observed dense local axon collateral system of the spiny neurons, was the realization that despite its three-dimensional shape, the striatum functions like a neuronal sheet: the shortest distance between cortex and basal ganglia output nuclei is just one corticostriatal synapse. Thus, the functional interaction between spiny projection neurons through local axon collaterals allowed the striatum to be viewed as a lateral feedback inhibitory network, where cortical inputs compete at the striatal level for control over basal ganglia outputs (e.g. Groves 1983; Wickens et al. 1991).

Hypotheses of striatal function based on lateral inhibition have been summarized as an instance of "winner-take-all" dynamics (Wickens et al. 1991), which is often implemented in neural networks for the unique selection among various alternatives. In its simplest form of a network with mutual inhibitory connections, the neuron that fires strongest will inhibit all surrounding neurons to which it is synaptically connected.

Despite these theoretical models and the ample neuroanatomical evidence of collateral innervation of spiny projection neurons, until recently there was little or no direct physiological evidence for functional synaptic inhibition among spiny projection neurons. The first attempts at paired recordings of nearby spiny neurons *in vivo* and *in vitro* failed to reveal synaptic connectivity (e.g., Jaeger et al. 1994). However, more recent studies from several groups have at last provided clear evidence for functional collateral synaptic connectivity among spiny projection neurons. Given this evidence, the existence of synaptic transmission between spiny projection neurons is no longer a question. Rather, the task now is to determine what the properties of the axon collateral synapses are and to infer the functional role of these connections in striatal signal processing. The precise form of that role will depend on factors like the amplitude of the synaptic response amplitude, the failure rate, short-term plasticity, and density of connectivity as well as the specific state of the postsynaptic neuron.

Amplitude Considerations

As with many neurons, membrane nonlinearity greatly complicates analysis of synaptic transmission in spiny projection neurons. As mentioned earlier, the

strong, fast inward rectifier, which maintains the hyperpolarized resting state of the spiny projection neurons *in vitro*, leads to a relatively low input resistance for these neurons as well as to a considerable electrotonic length. Compartmental models show that a strong shunting effect from this inward rectifier current at rest will result in considerable electrotonic degradation of the synaptic potential recorded at the soma compared to its amplitude at the site of origin on the dendrite. The shunting effect of intrinsic potassium currents is also demonstrated empirically by the finding that CsCl in the internal recording solution, which blocks most potassium currents, significantly increases somatically recorded peak values for synaptic PSPs and PSCs (see below). Finally, shunting effects can be, and almost certainly are, also introduced through the use of sharp intracellular electrodes.

Second, the position of the chloride reversal potential (E_{Cl-}) and the driving force for Cl^- ($E_m - E_{Cl-}$) in spiny projection neurons will largely affect the recorded synaptic peak values at the soma. For example, a synaptic peak current of ~ -30 pA, when $E_{Cl-} = -20$ mV, will shrink to an average peak current of ~ -10 pA, when $E_{Cl-} = -60$ mV, at a holding potential of ~ -80 mV. Because of the small diameter of spiny projection neuron dendrites, GABAergic activity during sustained neuronal activity could, in principle, change the internal chloride concentration in spiny projection neuron dendrites thereby changing the efficacy of the synaptic transmission.

With these considerations in mind, we summarize and attempt to compare the recently reported data on the collateral IPSP/IPSC below.

IPSP/IPSC Amplitude

Direct electrophysiological evidence of collateral inhibition among striatal spiny neurons was first demonstrated with dual sharp electrode recordings from pairs of spiny neurons in neostriatal slices. These recordings revealed extremely small IPSPs averaging about 0.25 mV in amplitude (Tunstall et al. 2002), or about one quarter the size of the IPSPs evoked from FS interneurons in spiny neurons with similar input resistances and similar postsynaptic membrane potentials (Koós and Tepper 1999).

Subsequently, paired whole-patch recordings found the amplitude of GABAergic IPSPs between spiny projection neurons to be between $\sim$1 and 3 mV in current clamp organotypic cortex–striatum–substantia nigra co-cultures or immature striatal slices (Czubayko and Plenz 2002). In the latter study, the recording conditions were different and input resistances of the spiny projection neurons were considerably higher than in the studies described above: 71 ± 5.2 MΩ (Tunstall et al. 2002); 67.4 ± 10.2 MΩ (Koós and Tepper 1999); 531 ± 4 MΩ in culture, 642 ± 4 MΩ in slice (Czubayko and Plenz 2002). This perhaps accounted, at least in part, for the reported differences in amplitudes of IPSPs.

Subsequently, a study in nucleus accumbens by Taverna et al. (2004) reported the PSP amplitudes of up to several mV between spiny projection

neurons with a mean IPSC amplitude of –31 ± 11 pA and peak conductance of g_{syn}= 0.6 nS (R_{in} = 195 ± 22 MΩ; E_{Cl-} = –20 mV). This is almost identical to those values found in mature organotypic striatal slice cultures, when correcting for differences in E_{Cl-} (11 ± 2 pA; Vm = –79 ± 1 mV; g_{syn}= 0.6 ± nS; E_{Cl-} = –60 mV; n = 10 neuronal pairs; Czubayko and Plenz, unpublished).

On the other hand, perforated patch recordings, which preserve native intracellular ion concentrations far better than whole-cell patch recordings, have revealed much lower peak IPSC values between 2–5 pA (Koós et al. 2002). Finally, experiments which include agents that fully or partially block potassium currents find increased peak IPSC values even when taking differences in E_{Cl-} into account: 67 ± 4 pA with QX-314 and 40 mM CsCl (Guzman et al. 2003); 130 ± 32 pA with 140 mM CsCl (Koós, Tepper and Wilson, unpublished).

These differences in experimental conditions (age, *in vitro* system, recording conditions, etc.) make it difficult to come up with a coherent picture about the functional role of the synaptic connection between spiny projection neurons. On the other hand, they might suggest important dynamic aspects of the synapse that go beyond experimental conditions alone. For example, differences seen with perforated patch recordings suggest that postsynaptic phosphorylation of the $GABA_A$ receptor might greatly control the strength of the synapse. Similarly, the dramatic increases in amplitude when potassium currents are blocked would imply that the postsynaptic membrane potential, which affects the anomalous rectification, might dynamically affect the impact of dendritic GABAergic input to control somatic spiking.

When similar methods of recording are used (e.g., Cs and high intracellular Cl^-), comparison of the FS–spiny IPSC with the spiny cell–spiny cell IPSC suggests that the former is approximately 4–5 times larger than the latter (Koós and Tepper 2002, Koós et al. 2002; Koós, Tepper, and Wilson, unpublished). A qualitatively similar but smaller difference was also suggested by Guzman et al. (2002). The difference in the sizes of the two IPSCs is consistent with the differential sites of synaptic contact of the two populations of GABAergic terminals. Intracellular labeling of spiny neurons revealed that when it was possible to identify the postsynaptic target of their local axon collaterals as a spiny neuron, 88% of the synapses were made onto interspine dendritic shafts and dendritic spines, with only 12% of the synapses ending on somata (Gerfen and Wilson 1996). This is in contrast to the sites of termination of parvalbumin-positive axons, which in addition to axodendritic synapses make pericellular baskets around the somata of spiny projection neurons (Bennett and Bolam 1994; Wilson 2004).

Failure Rate and Short-term Plasticity

All studies (Czubayko and Plenz 2002; Koós et al. 2002; Tunstall et al. 2002; Taverna et al. 2004) consistently report a failure rate of 30–50 % for the axon collateral synapse, much greater than that for the FS spiny cell synapse (> 1%;

Plenz and Kitai 1998; Koós and Tepper 1999, 2002). As might be expected, in two studies that tested for short-term plasticity, a pronounced facilitation/augmentation was found for synapses with high failure rate at a frequency above 10–15 Hz and a depression with a time course reminiscent of asynchronous release at or above 20 Hz for augmented responses (e.g., Figure 7.4b; Czubayko and Plenz 2002; Taverna et al. 2004). On the other hand, Koós et al. (2002) found a strong use-dependent depression reaching a steady-state amplitude of 30% or less of the initial amplitude during short train stimulations at 10 or 25 Hz. The depression recovered within 500 ms. The reason(s) for the apparent differences in short-term plasticity in these studies is not certain but could be due to differences in preparations and/or experimental conditions. If the short-term facilitation reported is dominant *in vivo*, this suggests that the connection is highly functional when spiny projection neurons burst intermittently, their preferred firing pattern *in vivo* in correlation with significant behavioral events.

Connectivity

These electrophysiological studies also shed some light on the connectivity between spiny projection neurons. The probability (P) was $P \cong 0.25$ in organotypic cultures for adjacent neurons at $D \cong 0$ (Czubayko and Plenz 2002), $P \cong 0.17$ in acute slices at $D \cong 0$–$50\ \mu m$ (Taverna et al. 2004), and $P = 0.10$–0.17 at $D \cong 50$–$100\ \mu m$ in the acute slice (Koós et al. 2002; Tunstall et al. 2002). Reciprocally connected pairs are exceedingly rare, being found in only one out of 122 pairs in three studies in acute slices from mature rats (Tunstall et al. 2002; Koós et al. 2002; Taverna et al. 2004). This low probability of reciprocal inhibition essentially eliminates striatal models that solely rely on mutual inhibition to encode mutually inhibitory actions to a subset of striatal neurons (Groves 1983; Wickens et al. 1991). This raises the question of what the specific role for asymmetrically connected spiny projection neurons might be.

Functional Role

These recent electrophysiological findings thus do not support the earlier views of the striatum as a lateral inhibitory network. Although there is no longer any doubt as to the existence of functional collateral inhibition, the characteristics of the synaptic response make it unlikely to mediate a "winner-take-all " type of scenario as proposed by many of the models. Recent quantal release experiments and modeling studies suggest that the IPSP recorded at the cell body is relatively small because each spiny neuron makes relatively few synapses with each other (i.e., N is about 3 compared to the FS spiny cell synapse, where N is about 7). In addition, each of these synapses is located relatively distally such that although the IPSP is small at the somatic recording site, it is considerably larger at its origin on the dendrite (Koós et al. 2004). If this is the case, then the

effects of the collateral IPSP may be of a more local nature, perhaps involving local dendritic integration or even possibly modulation of longer-term synaptic plasticity at individual synapses. Because of the nonlinear properties of spiny neurons, it is difficult to extrapolate accurately the strength of a synaptic connection measured at rest to the UP state. Thus far, recordings in cortex–striatum–substantia nigra co-cultures demonstrate that spiny neurons which are connected at rest also reveal a synaptic interaction during UP states in spike-triggered averages, as shown in Figure 7.2d.

The short-term facilitation reported suggests the lateral inhibition to be dominated by bursting spiny projection neurons. In addition, several factors further suggest a more complicated role of this synapse in striatal dynamics. First, the reversal potential of the chloride-mediated $GABA_A$ response is situated between the DOWN state membrane potential and spike threshold in spiny neurons. This allows the GABAergic synapse to be depolarizing or hyperpolarizing, depending on the membrane potential at the time of synaptic input. Second, recent findings on spike backpropagation in spiny projection neurons (Kerr and Plenz 2002) suggests that GABAergic synaptic transmission might affect synaptic plasticity by affecting the timing between action potential generation and incoming excitatory inputs. Whereas the relevance of many of these potential mechanisms to spiny neuron function remains to be determined, these recent findings underscore the potential complexity and richness of the functioning of the spiny cell recurrent collateral system, which remain to be worked out (Plenz 2003).

GABAergic Striatal Microcircuits Modulated by Dopamine

The striatum contains the highest density of tyrosine-hydroxylase immunoreactive fibers in the brain. These originate from the dopaminergic neurons of the substantia nigra, retrorubral field, and ventral tegmental area. Five dopamine (DA) receptors have been cloned that are grouped into a D_1-like family (D_1, D_5) and a D_2-like family (D_2, D_3, D_4). All receptors are present in the striatum and both families differ in the multitude of second messenger pathways they activate, with the D_1-like family acting through Gs/olf-stimulating adenylyl cyclase, and the D_2-like family acting through Gi/o-inhibiting adenylyl cyclase. As mentioned above, different DA receptor subtypes are largely segregated between spiny neurons projecting to the substantia nigra pars reticulata/internal pallidum that express mainly the D_1 receptor, and spiny neurons projecting to the external pallidum which express mainly the D_2 receptor. A subset (>20 %) of spiny projection neurons co-express DA receptors from both DA receptor families (Gerfen and Wilson 1996; Nicola et al. 2000).

The effects of DA on striatal function are enormously complex, in no small part because of the multiplicity of receptors and sites of action. Dopamine actions on spiny projection neurons appear to be predominantly or exclusively

modulatory in nature. That is, DA does not cause fast EPSPs or IPSPs that lead to simple excitation or inhibition in spiny neurons but rather acts to change the kinetics, activation, and inactivation voltage dependences and/or maximal conductances of a myriad of voltage-gated channels, including sodium, potassium, and calcium channels in the spiny neurons (Surmeier and Kitai 1993; Nicola et al. 2000).

Here we restrict our discussion to a few examples of the ability of DA to modulate synaptic transmission in striatal GABAergic microcircuits. Although many studies have demonstrated that DA modulates GABA responses in striatal spiny projection neurons, as one would expect, the results are complex and somewhat contradictory, which make it difficult to synthesize a coherent picture.

One report shows that D_1 receptor activation reduces postsynaptic currents evoked by local application of GABA in acutely dissociated neostriatal spiny projection neurons (Flores-Hernandez et al. 2000). In another, pharmacologically isolated GABAergic responses to intrastriatal stimulation were not modulated by local DA application in spiny projection neurons from striatum, but were in neurons from nucleus accumbens (Nicola et al. 2000). In contrast to this, Koós et al. (2002) reported that locally applied DA depressed the neostriatal spiny cell collateral IPSC by 63% in paired whole-cell recordings *in vitro*. Guzman et al. (2003) showed that GABAergic responses recorded from antidromic activation of striatal neurons in globus pallidus (presumably representing collateral IPSPs) were facilitated by D_1 agonists and inhibited by D_2 agonists, whereas a bicuculline-sensitive current evoked by intrastriatal stimulation (presumably reflecting feedforward inhibition from striatal interneurons) was not consistently modulated. Finally, D_2 receptor activation reduced pharmacologically isolated GABA responses to intrastriatal stimulation in about $\sim$30% of medium spiny neurons (Delgado et al. 2000). Some of these differences may arise from the source of the GABAergic input, that is, whether it derives from D_1 receptor expressing, D_2 receptor expressing spiny cell collaterals, or from interneurons.

Without direct effects on the membrane potential of spiny projection neurons, as mentioned above, DA does potently excite striatal parvalbumin-positive FS interneurons via activation of a D_1 receptor. At the same time, locally applied DA depresses the inhibition of these neurons by GABAergic inputs by acting on a presynaptic D_2 receptor (Bracci et al. 2002). Thus, despite the lack of a direct effect on the spiny cell membrane potential, DA may potently hyperpolarize and inhibit spiny projection neurons by acting through the FS interneurons, which exert a fast and strong synaptic inhibition on spiny projection neurons (Koós and Tepper 1999).

There has certainly been recent progress towards understanding the substrates of DA action in the striatum, because of the abundance of different $GABA_A$ receptor subunits in striatal neurons, the target specificity of short-term

plasticity as has been demonstrated for cortical GABAergic synapses, and the diversity of DA receptor in spiny projection neurons and interneurons. Still, a complete picture of dopaminergic modulation of intracellular GABAergic circuitry in the striatum lies in the future.

GABAergic Striatal Microcircuits Modulated by Acetylcholine

The levels of acetylcholine (ACh), choline acetyltransferase, and acetylcholinesterase are higher in striatum than in any other brain region. Similar to DA, despite being essential for normal striatal function, acetylcholine does not act upon spiny projection neurons to cause simple inhibition or excitation. Like DA, ACh modulates a number of voltage-gated channels in spiny projection neurons by activating muscarinic receptors; however, it does not by itself directly excite or inhibit the neuron.

Neurochemical studies reveal that cholinergic agonists increase basal striatal GABA overflow through nicotinic receptors *in vitro*. In contrast, electrically or potassium-stimulated GABA release is inhibited by muscarinic agonists (see references in Koós and Tepper 2002). Both effects can be explained by a recently described dual action of ACh on striatal FS interneurons.

The FS interneuron and the FS spiny cell synapse are both targets for independent cholinergic modulation. The FS interneuron is strongly depolarized by ACh acting through a nicotinic receptor. The excitation was blocked by mecamylamine but not by methyllycaconitine and was unaffected by CNQX and APV, indicating that it is mediated by a nicotinic receptor located postsynaptically on the FS interneuron, other than the rapidly desensitizing type 1 receptor (Koós and Tepper 2002). It is tempting to speculate as to a possible role for the FS interneuron in transducing the effects of rapid changes in cholinergic interneuron activity to the spiny projection neuron.

The GABA IPSP in spiny neurons evoked from FS interneurons was strongly suppressed (>80%) by ACh or muscarinic agonists. This modulation was mediated by pirenzapine-sensitive muscarinic receptors located presynaptically on the FS interneuron (Koós and Tepper 2002).

The contrasting neurochemical release studies can be well reconciled by these physiological results. FS interneurons are hyperpolarized and inactive *in vitro* and thus are unlikely to be releasing much GABA. Under these conditions there is not much GABA to be presynaptically inhibited and thus a cholinergic agonist would increase "basal" GABA release by stimulating the nicotinic receptors on the FS interneurons. Conversely, when FS interneurons are already firing and releasing GABA, additional cholinergic stimulation may produce only a minor further increase in firing rate and consequent GABA release and, therefore, the inhibitory presynaptic effect of the muscarinic receptors would predominate.

CONCLUDING REMARKS

A wealth of new data now exists on the functional microcircuitry of the neostriatum and this should, in the relatively near future, allow a much clearer understanding of how the neostriatum processes cortical and thalamic inputs en route to the basal ganglia output nuclei through which this system exerts powerful modulatory effects on a variety of essential voluntary motor and higher-order cognitive functions. Among the data that will figure prominently in the new syntheses of striatal function that will arise are the recent physiological studies of feedforward and feedback inhibition in the striatum, which show that these two GABAergic systems likely subserve very different roles in controlling striatal function. Rather than participating in a type of winner-take-all lateral inhibition, the spiny cell axon collaterals seem better suited towards controlling local dendritic function. The feedforward inhibition from the GABAergic interneurons, on the other hand, appear much more likely to be able to directly influence spike generation and timing.

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REFERENCES

Bennett, B.D., and J.P. Bolam. 1994. Synaptic input and output of parvalbumin-immunoreactive neurons in the neostriatum of the rat. *Neuroscience* **62**:707–719.

Bennett, B.D., and C.J. Wilson. 1999. Spontaneous activity of neostriatal cholinergic interneurons *in vitro*. *J. Neurosci*. **19**:5586–5596.

Bolam, J.P., and B.D. Bennett. 1995. Microcircuitry of the neostriatum. In: Neuroscience Intelligence Unit, Molecular and Cellular Mechanisms of Neostriatal Function, ed. M.A. Ariano and D.J. Surmeier, pp. 1–20. Austin: R.G. Landes.

Bracci, E., D. Centonze, G. Bernardi, and P. Calabresi. 2002. Dopamine excites fast-spiking interneurons in the striatum. *J. Neurophysiol.* **87**:2190–2194.

Courtemanche, R., N. Fujii, and A.M. Graybiel. 2003. Synchronous, focally modulated beta-band oscillations characterize local field potential activity in the striatum of awake behaving monkeys. *J. Neurosci.* **23**:11,741–11,752.

Czubayko, U., and D. Plenz. 2002. Fast synaptic transmission between striatal spiny projection neurons. *PNAS* **99**:15,764–15,769.

Delgado, A., A. Sierra, E. Querejeta, R.F. Valdiosera, and J. Aceves. 2000. Inhibitory control of the GABAergic transmission in the rat neostriatum by D_2 dopamine receptors. *Neuroscience* **95**:1043–1048.

Flores-Hernandez, J., S. Hernandez, G.L. Snyder et al. 2000. D_1 dopamine receptor activation reduces $GABA_A$ receptor currents in neostriatal neurons through a PKA/DARPP-32/PP1 signaling cascade. *J. Neurophysiol.* **83**:2996–3004.

Gerfen, C.R., and C.J. Wilson. 1996. The basal ganglia. In: Handbook of Chemical Neuroanatomy Vol 12: Integrated Systems of the CNS, Part III, ed. L.W. Swanson, A. Björklund, and T. Hökfelt, pp. 371–468. Amsterdam: Elsevier Science.

Graveland, G.A., and M. Difiglia. 1985. The frequency and distribution of medium-sized neurons with indented nuclei in the primate and rodent neostriatum. *Brain Res.* **327**:307–470.

Groves, P.M. 1983. A theory of the functional organization of the neostriatum and the neostriatal control of voluntary movement. *Brain Res.* **5***:*109–132.

Guzman, J.N., A. Hernandez, E. Galarraga et al. 2003. Dopaminergic modulation of axon collaterals interconnecting spiny neurons of the rat striatum. *J. Neurosci.* **23**:8931–8940.

Jaeger, D., H. Kita, and C.J. Wilson. 1994. Surround inhibition among projection neurons is weak or nonexistent in the rat neostriatum. *J. Neurophysiol.* **72**:1–4.

Kawaguchi, Y. 1993. Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *J. Neurosci.* **13**:4908–4923.

Kawaguchi, Y., C.J. Wilson, S.J. Augood , and P.C. Emson. 1995. Striatal interneurons: Chemical, physiological, and morphological characterization. *TINS* **18**:527–535.

Kerr, J.N., and D. Plenz. 2002. Dendritic calcium encodes striatal neuron output during up states. *J. Neurosci.* **22**:1499–1512.

Kita, H. 1993. GABAergic circuits of the striatum. *Prog. Brain Res.* **99**:51–72.

Kita, H., and S.T. Kitai. 1988. Glutamate decarboxylase immunoreactive neurons in rat neostriatum: Their morphological types and populations. *Brain Res*. **447**:346–352.

Kita, H., T. Kosaka, and C.W. Heizmann. 1990. Parvalbumin-immunoreactive neurons in the rat neostriatum: A light and electron microscopic study. *Brain Res.* **536**:1–15.

Koós, T., and J.M. Tepper. 1999. Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nat. Neurosci.* **2**:467–472.

Koós, T., and J.M. Tepper. 2002. Dual cholinergic control of fast-spiking interneurons in the neostriatum. *J. Neurosci.* **22**:529–535.

Koós, T., J.M. Tepper, P. Goldman-Rakic, and C.J. Wilson. 2002. Electrophysiological properties and dopaminergic modulation of GABAergic inhibition among neostriatal projection neurons. *Soc. Neurosci. Abstr.* **28**:764.17.

Koós, T., J.M. Tepper, and C.J. Wilson. 2004. Comparison of IPSCs evoked by spiny and fast-spiking neurons in the neostriatum. *J. Neurosci.* **24**:7916–7922.

Nicola, S.M., D.J. Surmeier, and R.C. Malenka. 2000. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Ann. Rev. Neurosci.* **23**:185–215.

Plenz, D. 2003. When inhibition goes incognito: Feedback interaction between spiny projection neurons in striatal function. *TINS* **26**:436–443.

Plenz, D., and S.T. Kitai. 1998. "Up" and "down" states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fast-spiking interneurons studied in cortex–striatum–substantia nigra organotypic cultures. *J. Neurosci.* **18**:266–283.

Rymar, V.V., R. Sasseville, K.C. Luk, and A.S. Sadikot. 2004. Neurogenesis and stereological morphometry of calretinin-immunoreactive interneurons of the neostriatum. *J. Comp. Neurol.* **469**:325–339.

Surmeier, D.J., and S.T. Kitai. 1993. D_1 and D_2 dopamine receptor modulation of sodium and potassium currents in rat neostriatal neurons. *Prog. Brain Res*. **99**:309–324.

Taverna, S., Y.C. Van Dongen, H.J. Groenewegen, and C.M. Pennartz. 2004. Direct physiological evidence for synaptic connectivity between medium-sized spiny neurons in rat nucleus accumbens *in situ*. *J. Neurophysiol.* **91**:1111–1121.

Tepper, J.M., N.A. Sharpe, T.Z. Koós, and F. Trent. 1998. Postnatal development of the rat neostriatum: Electrophysiological, light- and electron-microscopic studies. *Dev. Neurosci*. **20**:125–145.

Tunstall, M.J., D.E. Oorschot, A. Kean, and J.R. Wickens. 2002. Inhibitory interactions between spiny projection neurons in the rat striatum. *J. Neurophysiol.* **88**:1263–1269.

Wickens, J.R., M.E. Alexander, and R. Miller. 1991. Two dynamic modes of striatal function under dopaminergic-cholinergic control: Simulation and analysis of a model. *Synapse* **8**:1–12.

Wilson, C.J. 2004. Basal ganglia. In: The Synaptic Organization of the Brain, 5 ed, ed. G.M. Shepherd, pp. 361–414. Oxford: Oxford Univ. Press.

8

Modulation of Striatal Circuits by Dopamine and Acetylcholine

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ABSTRACT

An adaptive organism must be able to predict future rewarding and aversive events and to modify its behavior and predictions according to the mismatch between these predictions and reality. A major class of artificial systems that can optimize their behavior in a complex and only partially predictable environment is the class of actor/critic machines. In these machines, the critic is intended to predict the cumulative sum of reinforcement produced by the environment, whereas the actor chooses the action that would maximize this sum. Recent computational models of the basal ganglia call attention to this family of actor/critic models of machine reinforcement learning for two main reasons: (a) the similarity between the critic's error signal and the dopaminergic message of mismatch between prediction and reality, and (b) the dopamine and cholinergic modification of the efficacy of the corticostriatal synapses. This chapter highlights the basal ganglia architecture as an actor/critic reinforcement learning network and reviews the physiological literature (extracellular recording in behaving animal) of the teaching signals of basal ganglia dopaminergic and cholinergic neurons, and the effects of dopamine and acetylcholine on striatal synapses.

REINFORCEMENT LEARNING NETWORKS AND THE BASAL GANGLIA

The field of classical machine learning theories traditionally divides the term "learning" into two separate categories. In supervised learning, the learning element—the agent—is accompanied by an all-knowing element—the teacher—which informs it on the correct action to be taken in each situation. Unsupervised learning lacks the all-knowing teacher element. In this case, only the inputs are used and classified according to the network dynamics. These

unsupervised systems are self-organizing, usually applied in tasks aimed at discovering regularities in input statistics, and do so by adjusting weights (synaptic efficacies) according to some local learning rule (Hebb 1949). Application of these two learning modes to modeling of the basal ganglia encounters some feasibility problems. The most obvious one relates to supervised learning, since introducing an all-knowing teacher to a network is biologically unrealistic. At the other extreme, classical unsupervised learning (e.g., the original Hopfield network or principal component analysis network) will never discover critical information that does not correspond to the statistical structure of the input (Dayan and Abbott 2001), no matter if this information or behavior is reinforced or punished by the past and present history of the organism.

A combination of both learning categories is reinforcement learning. The field of reinforcement learning generally deals with situations in which an agent with an explicit goal acts upon the environment. The agent's actions change the state of the environment, which in turn provides feedback (reward, punishment, or null) to the agent on its actions. In this scheme, external reward functions as an evaluative signal, indicating the degree of appropriateness of network performance. Thus, on one hand, reinforcement learning is a form of supervised learning, because the network receives and uses feedback information from the environment. However, this information is evaluative, rather than instructive, and the correct answer itself remains unknown to the actor. Reinforcement learning is therefore sometimes referred to as weakly or minimally supervised learning.

An ideal reinforcement learning system chooses a policy (e.g., a set of stimulus–response rules or associations) by evaluating the value of each of its alternatives. The value of an alternative is defined as the (weighted) sum of rewards received when starting in that state and following a fixed policy in all the future states. However, to do so we need a method for predicting future returns (rewards) for a course or a sequence of states. Temporal difference (TD) models (Sutton and Barto 1998) are a class of models that address this task. In each step, the state value is compared with the immediate reward plus the estimated value of the next state. The result of this comparison, called the TD error, is used to update the state value. Thus, TD is naturally implemented on-line, since it is truly incremental and the state value is updated only after a single step. The TD algorithm uses the actual reward as well as the difference between the present and past reward predictions as the error signal for updating its prediction (critic) and behavior (actor, see below). Initially, we may be surprised by how an incorrect prediction can be improved by moving it towards another incorrect prediction: in A. Barto's words (1995), "it is the blind leading the blind." The key observation of Sutton and Barto (1998) is that future prediction tends to be better than the current prediction because it includes the additional data provided by the present reward; that is, more like the blind being led by the slightly less blind (Barto 1995). Over time, this algorithm will converge to the correct prediction.

In animal and human learning as well as in machine learning, reward is often delayed relative to the behavior that caused it. Thus, the actor must perform a

sequence of actions before it receives information regarding the correctness of the response. Therefore, a frequent difficulty in reinforcement learning is that of temporal credit assignment. To each action in the sequence, the actor must be able to assign credit or blame individually. The actor/critic architecture provides reinforcement learning with a solution to the temporal credit assignment problem (Barto 1995). In this architecture, a separate unit (critic) receives from the environment all the information that the agent (actor) receives; that is, the inputs and reinforcement, along with information regarding the actor's output. The critic is intended to predict the reinforcement produced by the environment. Since the actor should try to maximize the cumulative reinforcement, rather than the immediate reward, a weighted average of the future reinforcement is estimated and fed back, step by step, by the critic to the actor. A network (or algorithm) used to predict reinforcement and feed the difference between its predictions and true reinforcement to the actor is called the adaptive critic (Sutton and Barto 1998), and the entire system is termed actor/critic. The temporal credit assignment problem is solved, since the critic provides the actor with immediate evaluative feedback. The term "adaptive" emphasizes the fact that the critic adapts (according to its expectations) upcoming rewards to immediate evaluative feedback.

The basic idea of the actor and the critic learning rule is that if an action (or sequence of actions) produced in response to a sensory input has resulted in the expected outcome (a match between prediction and reality), then the stimulus–response association remains unchanged. However, if the consequences are better or worse than predicted, then the stimulus–response association is strengthened or weakened, respectively. The TD error equals the difference between prediction and reality, and therefore can be used as the teaching signal for the actor/critic network. The adaptive critic uses the TD error to update the weights of the "synaptic inputs" to the critic "neurons." In fact, the synaptic efficacies of the actor are modified by the same TD error as well. This is reasonable if one considers the actor's learning objective of maximizing future cumulative rewards. If a response to a sensory stimulus has the expected consequences (TD error = 0), then that response tendency should remain unchanged. However, if the consequences are either better (TD error is positive) or worse (TD error is negative) than expected, the response should be strengthened or weakened accordingly. In most cases, a modified triple Hebbian rule (synaptic efficacy is modified according to the coincidence of the reinforcement signal and pre- and postsynaptic activity) is used for updating of the efficacies of the synapses (Bar-Gad and Bergman 2001).

There are several important questions and controversial issues: Is the "mismatch between prediction and reality" the optimal teaching signal? Should we implement motivation, attention, or context dependency into the TD model (Dayan and Balleine 2002; Nakahara et al. 2002)? Computationally, these questions are transformed into the question of the effects of several teachers with partially overlapping/partially conflicting messages to the learning network. The

future discounting factor plays a major role in balancing the network behavior regarding close and remote rewards. Is this discounting factor fixed, or may it be modified by one of the teaching signals or the network inputs? Further, what is the coding strategy of the basal ganglia network? Is it a "winner-take-all" situation (Mink 1996) or more broad coding, as suggested by the reinforcement-driven dimensionality reduction model (Bar-Gad et al. 2003)? Are these coding schemes shared by all the basal ganglia networks, or might the basal ganglia be composed of segregated independent channels of actors? Finally, the complementary roles of other brain learning networks (e.g., the cerebellum, frontal cortex) should be evaluated in parallel with those of the basal ganglia (Doya 2000, 2002).

Ultimately, the aim of a reinforcement learning system is to maximize the expected return, which is a function of the subsequent reward sequence. Since the reinforcement signal gives no hint as to what the correct answer should be, a reinforcement learning network must include a source of randomness to enable exploration of the set of all possible outputs until an optimal one is found. This notion embodies a key element in reinforcement learning strategy: exploration versus exploitation. The agent has to exploit what it already knows to obtain a reward, but it also has to explore to achieve better performance in the future. A proper trade-off between exploration and exploitation is a key question in policy or life-strategy selection. Thus, a key question in basal ganglia research is: What is the source of the randomness signal in this learning network (K. Doya, pers. comm.)? We hope that the following sections, which indicate the major similarities between the basal ganglia and reinforcement learning actor/critic network, will encourage you to explore this family of new models of the basal ganglia (Houk et al. 1995; Suri and Schultz 2001).

ROLES OF REPRESENTATION AND PROCESSING OF REINFORCEMENT AND MOTIVATION BY DOPAMINE NEURONS AND STRIATAL NEURONS

Dopamine Neurons

Dopamine-containing neurons have their cell bodies in the midbrain, A8-A9-A10 cell complex of Dahlström and Fuxe, and broadly innervate the dorsal and ventral striatum, limbic structures, and the frontal cerebral cortices through their axons with varicosities. In most actor/critic models of the basal ganglia, the dopamine neurons play the role of the critic. Roles of the dopaminergic innervation in planning, initiation, and control of movements as well as in learning of actions were suggested based on the deficits in Parkinson's disease patients and in experimental animals with lesions in the dopamine system. Inactivation of dopamine innervation of the nucleus accumbens suggested roles in motivation. The most effective structures for intracranial self-stimulation are the medial

forebrain bundle containing ascending axons of dopamine neurons and the cell area of dopamine neurons. Certain major drugs of abuse are related intimately to dopaminergic transmission. Finally, in the human brain, regions belonging to a mesostriatal and mesolimbic dopamine system respond to reinforcement (Martin-Soelch et al. 2001; Thut et al. 1997).

Recordings of activity of dopamine neurons have provided information of how the dopamine system participates in reward, motivation, and learning mechanisms. Dopamine neurons in alert rats, cats, and monkeys tonically discharge at about 4 to 5 spikes/second. They increase their discharges when food or liquid rewards are delivered. Although the presentation of nonreward objects does not usually influence dopamine neuron activity, they become effective to activate phasically when they appear as novel, high-intensity stimuli, and when they were associated with rewards. Dopamine neurons do not seem to be sensitive to stimuli of an aversive nature, such as air puff to the animal's face (Mirenowicz and Schultz 1996). On the other hand, tonic discharge rate of lightly anesthetized rat decreased by aversive tail pinch (Ungless et al. 2004).

A behavior followed by rewards tends to occur again in the same context because of positive reinforcement of the behavior by the rewards. Thus, well-earned rewards let subjects stay in the behavior, whereas rewards that appear unexpectedly change behavior. Recordings of dopamine neuron activity in behaving monkeys revealed that unpredictability of the rewards is the critical feature for neuronal responses. Unexpectedly delivered rewards phasically activate dopamine neurons, whereas activation after the rewards does not occur if reward-predictive stimuli precede the rewards (Schultz et al. 1997). Based on this observation, a hypothesis was proposed that dopamine neurons code reward-prediction errors. The hypothesis was tested in more elaborate paradigms: magnitudes of activation of dopamine neurons after reward were negatively correlated with probability of reward in the classical and instrumental conditioning paradigms (Fiorillo et al. 2003; Morris et al. 2004). Precise encoding of reward-expectation errors was directly demonstrated in a voluntary decision task under probabilistic reward paradigm (Satoh et al. 2003). Dopamine neurons can code reward-prediction errors that are dependent upon the behavioral context (reward history in preceding trials) (Nakahara et al. 2004). These experimental demonstrations of coding reward-prediction error are important because this is consistent with the proposed role of TD error signal in reinforcement learning theories as described above. Reward-predicting stimuli evoke phasic activation of dopamine neurons, which may reflect subject's reward expectation. On the other hand, it was recently shown that the magnitudes of neuronal responses to reward-predicting stimuli appear to represent motivational properties rather than reward expectation per se, because their magnitude at trials with identical reward expectation had significant negative correlation with reaction times of animals after the stimuli (Satoh et al. 2003). Encoding of motivation by dopamine neurons is consistent with well-documented responses to a wide variety of

salient sensory events, including novel and high-intensity stimuli. This might be a neural substrate for coding incentive salience proposed by motivational theories.

Although knowledge about dopamine neuron activity has accumulated rapidly, questions on several issues have not been addressed and thus remain unanswered:

1. How do the dopamine neurons compute reward-prediction errors? Through which neural circuits do they receive signals for the computation? Currently proposed loci are the dorsal striatum, ventral striatum, and pedunculopontine tegmental nucleus (PPTN).
2. How can the coding of motivation and biologically salient stimuli by dopamine neurons be reconciled with reinforcement learning theories?
3. Are there any differences in represented information of dopamine neurons among A8, A9, and A10 cell groups?
4. What are the roles of recently observed uncertainty-related tonic activation of dopamine neurons (Fiorillo et al. 2003) in learning and selection of action?

Striatal Neurons

In animals performing behavioral tasks, GABAergic projection neurons have a very low rate of background discharges (usually less than 0.2 spikes/s), show burst discharges of action potentials, and are thus called phasically active neurons (PANs). Presumed cholinergic interneurons have tonic discharges at about 2–8 spikes/s and are thus called tonically active neurons (TANs); however, they never show spontaneous burst discharges. TANs were found initially to respond to stimuli associated with reward in terms of characteristic pause usually followed by facilitation of discharges. Responses to rewards or reward-predictive stimuli seem to be larger when presented in an unpredicted manner, as in the case of dopamine neurons (Ravel et al. 2001). On the other hand, subsequent studies revealed that they respond to aversive stimuli as well, such as air puff to animal's face (Blazquez et al. 2002), although temporal patterns of the responses vary (Ravel et al. 2003). TAN responses show a high correlation with behavioral outcomes (blinks) in such paradigms (Blazquez et al. 2002). TANs in the caudate nucleus of monkeys performing saccadic eye movement task do not respond to reward-predicting visual cues, although they respond to instructions for saccade direction presented at contralateral visual field. This apparent inconsistency in observations was addressed recently in a task in which monkeys released a lever using their hands with three different motivational outcomes: reward, aversive air puff on the face, and neutral sound (Yamada et al. 2004). TANs selectively respond to visual stimuli instructing motivational outcomes. Most TANs discriminate the three kinds of instructions. A higher percentage of TANs in the caudate nucleus respond to stimuli associated with motivational outcomes than in the

putamen, whereas a higher percentage of TANs in the putamen respond to GO signals for lever release than in the caudate nucleus, especially under reward context. This may be why most TANs recorded in the caudate nucleus of monkeys performing saccade tasks were similarly responsive to reward-associated and no-reward-associated visual instructions preceding the GO signal. It is, thus, indicated that TANs encode instructed motivational contexts by responding selectively and by discriminating the stimuli associated with motivational outcomes.

PANs (the medium spiny neurons of the striatum and part of the "actor" in the actor/critic model of the basal ganglia) exhibit burst discharges in relation to sensory stimuli, body and eye movements, reward, and expectation of these events depending on the location of neurons in the striatum, which receives specific cortical projections through the corticobasal ganglia loops. Primary feature of the activity is its strong modification by the reward predictability. For example, magnitudes of responses to sensory cues and of movement-related activity change drastically by the reward predictability. Changes of preferred direction of neuronal activity occur rapidly. Most PANs show quantitatively larger activation when larger reward is expected, although smaller numbers of PANs show larger activation when smaller reward is expected. The expected gain of reward influences behavioral responses, response bias, and some PANs in the monkey caudate nucleus, and this seems to code neuronal signals for response bias by exhibiting tonic increase of discharges before appearance of reward-predictive cue (Lauwereyns et al. 2002). Striatal neurons adaptively change their activity based on the reward predictability through sensorimotor association learning (Schultz et al. 2003) and habit learning (Jog et al. 1999). These reward-dependent, short- and long-term flexibilities of neuronal representation and processing are the basis for the striatum to play central roles in reward-based decision making and learning, and are accomplished by dopamine-dependent modification of synaptic transmissions in PANs, TANs, and other interneurons (Wickens et al. 2003; see also below). Similarities and differences in dopaminergic and cholinergic actions in the neuronal circuits of the striatum provides important clues for understanding the circuit design of the striatum (Graybiel et al. 1994; Morris et al. 2004).

Several important questions and controversial issues arise: First, how do the signals of reward-expectation errors and motivation conveyed by dopamine neurons contribute to the short- and long-term flexibility of striatal neuron activity? Second, how do TANs modify activity of PANs? What are the roles of microcircuit neurons in the striatum, such as GABAergic and somatostatin-containing interneurons, for intrastriatal information processing? What kind of roles does the presumed functional macro-unit in the striatum, striosomes, and within the matrix, matrisomes, play in the reward-based adaptive representation of motor and cognitive signals? Striosomes, for example, have been suggested to serve the role of the adaptive critic in the actor/critic model of Sutton and

Barto (Houk et al. 1995). Third, dopamine-dependent synchronization and oscillation of neuronal activity was found in the globus pallidus (Heimer et al. 2002) and striatum (Courtemanche et al. 2003). Does the characteristic activity originate from the same basic mechanism as oscillatory activity in the striatum and globus pallidus–subthalamus network? What are their roles in the processing of the basal ganglia?

CELLULAR PHYSIOLOGICAL STUDIES OF MODIFICATION OF THE EFFICACY OF BASAL GANGLIA SYNAPSES

The critic in actor/critic reinforcement learning network has to code (respond to) the mismatch between prediction and reality (TD error) and to modify the synaptic efficacies of the actor network. Above, we discussed learning-related changes in response properties of three main sets of neurons: substantia nigra/ventral tegmental area dopamine neurons, cholinergic interneurons (TANs), and spiny projection neurons (PANs). Two interrelated questions arise: What is the cellular mechanism underlying these changes in response properties? What are the downstream consequences of these changes in response properties for the neurons that they innervate? To address these questions we need to consider the consequence of the altered firing patterns of dopamine cells and cholinergic interneurons for information processing by spiny projection neurons. In particular, we need to consider their effects on the efficacy of glutamatergic inputs from the cerebral cortex and thalamus to striatal spiny projection neurons, and the excitability of the spiny projection neurons as determined by the spectrum of ion channels modulated by dopamine and acetylcholine. These effects are both time and activity dependent. While detailed consideration of these effects leads to a complex and almost intractable network of interactions, we hypothesize an underlying purpose, namely, to implement rules for modification of synaptic efficacy as required by a reinforcement learning system with a final goal of maximizing the probability of actions that lead to favorable outcomes. The question then becomes: What are the rules of synaptic modification in the basal ganglia?

Synaptic Plasticity in the Striatum

Synaptic plasticity is a change in the functional efficacy of synaptic connections that is induced by certain patterns of brain activity. For computational purposes, both short- and long-lasting changes are required. Also important for computational purposes, the precise requirements for induction of synaptic plasticity to represent a "rule" for synaptic modification, which in principle should be capable of being formulated as a mathematical equation with several variables or factors. Hebbian, or two-factor rules, have been studied extensively in cortical and hippocampal circuits. However, the logic of reinforcement learning requires an

additional "teaching" input. Reward-related activity in nigrostriatal dopamine projections and reward-dependent modification of striatal cell responses suggest that dopamine afferents, and probably other neuromodulators such as acetylcholine, are involved as such a teaching input in the changes in neural responses that occur in the neostriatum in association with learning. Dopamine-dependent modulation or plasticity of the corticostriatal and thalamostriatal pathways is a probable basis for the learning-related changes in neuronal responses measured in single-unit studies in behaving animals. The underlying cellular mechanisms are best understood in relation to learning-related changes in spiny projection neuron activity, where synaptic plasticity in the corticostriatal pathway seems to play an important role.

The spiny projection neurons receive excitatory synaptic inputs from the cortex and thalamus. Action potential firing activity in spiny projection neurons requires this excitatory drive (Wilson et al. 1983). Thus, changes in the afferent activity or efficacy of these corticostriatal and thalamostriatal inputs probably underlie learning-related changes in striatal cell activity.

Long-term depression (LTD) can be induced in the corticostriatal synapses by high-frequency stimulation (HFS) of the cerebral cortex. It is a depolarization-dependent process that requires activation of voltage-sensitive calcium channels in the postsynaptic cell during the conditioning HFS, as well as an increase in intracellular calcium concentration (Bonsi et al. 2003). These conditions are likely to be met in striatal cells, which fire action potentials in response to excitatory synaptic input (Kerr and Plenz 2002).

Long-term potentiation (LTP) has also been reported in the striatum. Initial reports of striatal LTP were based on the effects of HFS in slices bathed in magnesium-free fluid (Calabresi et al. 1992). Both dopamine depletion and the dopamine D_1 receptor antagonists block LTP in magnesium-free fluid (Kerr and Wickens 2001). Conversely, dopamine, applied in a manner which mimics the natural release of dopamine produced by reward, is sufficient to facilitate LTP (Wickens et al. 1996). In these latter experiments, dopamine was applied in brief pulses coinciding with a pre- and postsynaptic conjunction of activity. The pulsatile application of dopamine reversed the LTD which normally follows HFS, and potentiation of responses was induced.

The use of intracellular recording in whole-animal preparations has enabled greater separation of stimulating electrodes and more specific activation of afferents than is possible in brain slices. Using this method, HFS of the cerebral cortex induces LTD of the corticostriatal pathway, as in slices. When low-frequency stimulation of the substantia nigra pars compacta is paired with cortical HFS, a short-lasting potentiation is induced (Reynolds and Wickens 2000). This short-lasting potentiation is blocked by dopamine depletion. Experiments using extracellular single-unit recordings of nucleus accumbens neurons in combination with chronoamperometric measures of dopamine efflux have led to a similar conclusion (Floresco et al. 2001). Using substantia nigra intracranial self-

stimulation (ICSS) as a model for reward-related learning, Reynolds et al. (2001) measured responses to cortical afferents before and after ICSS-like stimulation of the substantia nigra dopamine cells. Stimulation of the substantia nigra with behaviorally reinforcing parameters induced potentiation of corticostriatal synapses. In addition, the degree of potentiation up to 10 minutes after the stimulus trains was correlated with the rate of learning of ICSS. Animals showing a greater degree of potentiation were correspondingly faster to reach criteria for ICSS, and vice versa. Potentiation was blocked in control animals administered a dopamine D_1-like receptor antagonist. These findings suggest that stimulation of the substantia nigra may positively reinforce behavior by dopamine D_1 receptor-dependent potentiation of cortical inputs to the striatal spiny neurons.

These findings support the hypothesis of a three-factor rule for synaptic modification in the striatum. However, very little is known of the precise nature of this rule. Each of the three factors (presynaptic activity, postsynaptic depolarization, dopamine concentration) has temporal and magnitude characteristics, which may influence the extent and direction of changes in synaptic efficacy. Further studies are needed to measure the effects of extent and exact timing of the dopamine concentration changes in relation to the other two factors.

Other neuromodulators are also likely to be involved in synaptic modification rules. Acetylcholine may modulate synaptic plasticity at corticostriatal synapses via muscarinic (Calabresi et al. 1998) or nicotinic (Partridge et al. 2002) actions. Acetylcholine is released across the entire striatal network by striatal cholinergic interneurons. These interneurons, as noted previously, also exhibit learning-related changes in activity. This is not an immediate effect of changes in dopamine afferent activity, but may involve dopamine-dependent plasticity of inputs.

Dopamine-dependent synaptic plasticity has been described in the striatal cholinergic interneurons (Suzuki et al. 2001). Stimulation of the corticostriatal and thalamostriatal afferents with single pulses produced a depolarizing and hyperpolarizing postsynaptic potential, thought to reflect an initial excitation by the stimulated afferents followed by an inhibitory postsynaptic potential mediated by intrastriatal collaterals of the spiny projection neurons. HFS of the excitatory afferents produced dopamine-dependent LTP of the excitatory postsynaptic potential, and an increase in the probability of the disynaptically mediated inhibitory potential (Suzuki et al. 2001). These two effects may combine in the acquisition of the pause responses of the cholinergic interneurons, as described above (Aosaki et al. 1994).

Short-term Modulation of Striatal Circuitry

In addition to the effects of dopamine on the induction of synaptic plasticity, there are many immediate short-term effects of dopamine mediated by voltage-

and receptor-operated channels (Nicola et al. 2000). These effects depend on the membrane potential of the postsynaptic cell and its recent history because these variables determine the state of the channels modulated by dopamine. A transient increase in dopamine concentration may interact with the postsynaptic cell in a state-dependent way. For example, in hyperpolarized neurons, increasing the inward rectifier current by D_1 receptor activation holds cells hyperpolarized. In depolarized neurons, D_1 receptor activation increases non-inactivating calcium currents, and this favors maintained depolarization. The functional significance of such a mechanism may be to produce a short-term increase in the gain of selected corticostriatal pathway circuits that may facilitate approach to rewarding stimuli. This may be a mechanism by which the acquisition of dopamine cell firing responses to incentive stimuli is translated into approach behavior.

CONCLUSION

There are major similarities between the basal ganglia and reinforcement learning actor/critic networks. Dopamine neurons respond in ways that might encode reward-prediction errors, as required of the teaching signal in reinforcement learning models. The longer-term effects of dopamine on corticostriatal synaptic input to striatal spiny projection neurons are consistent with three-factor rules required for reinforcement learning. There is also evidence that dopamine neurons represent motivational properties and may serve as a neural substrate to encode incentive salience. The more immediate and reversible actions of dopamine may be linked to initiation of movements, brought about by facilitation of striatal output by anticipatory firing of dopamine cells in response to incentive cues. Cholinergic interneurons, or TANs, encode instructed motivational contexts. The input to TANs is also subject to regulation by dopamine. The output of TANs may, in turn, modulate the effects of the dopamine signal, as well as have direct effects on the spiny projection neurons. There is evidence to support, in principle, the basic idea that dopamine and acetylcholine are involved in the activation of selected corticostriatal pathway circuits by incentive stimuli and strengthening by positive reinforcement. Many important questions and controversial issues remain: How good is the agreement between the predictions of machine learning theory regarding the teaching signal and neural activity in dopamine and acetylcholine neurons? In the context of the basal ganglia, what is encoded in the inputs and outputs from the striatum, which in machine learning terms correspond to the stimulus and response? Finally, how well do the cellular actions of dopamine and acetylcholine translate into the learning rules required for adaptive actor/critic behavior?

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REFERENCES

Aosaki, T., A.M. Graybiel, and M. Kimura. 1994. Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. *Science* **265**:412–415.

Bar-Gad, I., and H. Bergman. 2001. Stepping out of the box: Information processing in the neural networks of the basal ganglia. *Curr. Opin. Neurobiol*. **11**:689–695.

Bar-Gad, I., G. Morris, and H. Bergman. 2003. Information processing, dimensionality reduction, and reinforcement learning in the basal ganglia. *Prog. Neurobiol*. **71**: 439–473.

Barto, A.G. 1995. Adaptive critics and the basal ganglia. In: Models of Information Processing in the Basal Ganglia, ed. J.C. Houk, J.L. Davis, and D.G. Beiser, pp. 215–232. Cambridge, MA: MIT Press.

Blazquez, P.M., N. Fujii, J. Kojima, and A.M. Graybiel. 2002. A network representation of response probability in the striatum. *Neuron* **33**:973–982.

Bonsi, P., T. Florio, A. Capozzo et al. 2003. Behavioural learning-induced increase in spontaneous GABAA-dependent synaptic activity in rat striatal cholinergic interneurons. *Eur. J. Neurosci*. **17**:174–178.

Calabresi, P., D. Centonze, P. Gubellini, A. Pisani, and G. Bernardi. 1998. Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. *Eur. J. Neurosci.* **10**:3020–3023.

Calabresi, P., A. Pisani, N.B. Mercuri, and G. Bernardi. 1992. Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of NMDA receptor channels. *Eur. J. Neurosci.* **4**:929–935.

Courtemanche, R., N. Fujii, and A.M. Graybiel. 2003. Synchronous, focally modulated β-band oscillations characterize local field potential activity in the striatum of awake behaving monkeys. *J. Neurosci.* **23**:11,741–11,752.

Dayan, P., and L.F. Abbott. 2001. Theortical Neuroscience, Computational, and Mathematical Modeling of Neural Systems. Cambridge, MA: MIT Press.

Dayan, P., and B.W. Balleine. 2002. Reward, motivation, and reinforcement learning. *Neuron* **36**:285–298.

Doya, K. 2000. Complementary roles of basal ganglia and cerebellum in learning and motor control. *Curr. Opin. Neurobiol.* **10**:732–739.

Doya, K. 2002. Metalearning and neuromodulation. *Neural Netw.* **15**:495–506.

Fiorillo, C.D., P.N. Tobler, and W. Schultz. 2003. Discrete coding of reward probability and uncertainty by dopamine neurons. *Science* **299**:1898–1902.

Floresco, S.B., C.D. Blaha, C.R. Yang, and A.G. Phillips. 2001. Dopamine D1 and NMDA receptors mediate potentiation of basolateral amygdala-evoked firing of nucleus accumbens neurons. *J. Neurosci*. **21**:6370–6376.

Graybiel, A.M., T. Aosaki, A.W. Flaherty, and M. Kimura. 1994. The basal ganglia and adaptive motor control. *Science* **265**:1826–1831.

Hebb, D.O. 1949. The Organization of Behavior. New York: Wiley.

Heimer, G., I. Bar-Gad, J.A. Goldberg, and H. Bergman. 2002. Dopamine replacement therapy reverses abnormal synchronization of pallidal neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primate model of Parkinsonism. *J. Neurosci.* **22**:7850–7855.

Houk, J.C., J.L. Adams, and A.G. Barto. 1995. A model of how the basal ganglia generate and use neural signals that predict reinforcement. In: Models of Information Processing in the Basal Ganglia, ed. J.C. Houk, J.L. Davis, and D.G. Beiser, pp. 249–270. MIT Press.

Jog, M.S., Y. Kubota, C.I. Connolly, V. Hillegaart, and A.M. Graybiel. 1999. Building neural representations of habits. *Science* **286**:1745–1749.

Kerr, J.N., and D. Plenz. 2002. Dendritic calcium encodes striatal neuron output during up-states. *J. Neurosci.* **22**:1499–1512.

Kerr, J.N., and J.R. Wickens. 2001. Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum *in vitro*. *J. Neurophysiol*. **85**: 117–124.

Lauwereyns, J., K. Watanabe, B. Coe, and O. Hikosaka. 2002. A neural correlate of response bias in monkey caudate nucleus. *Nature* **418**:413–417.

Martin-Soelch, C., A.F. Chevalley, G. Kunig et al. 2001. Changes in reward-induced brain activation in opiate addicts. *Eur. J. Neurosci.* **14**:1360–1368.

Mink, J.W. 1996. The basal ganglia: Focused selection and inhibition of competing motor programs. *Prog. Neurobiol*. **50**:381–425.

Mirenowicz, J., and W. Schultz. 1996. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* **379**:449–451.

Morris, G., D. Arkadir, A. Nevet, E. Vaadia, and H. Bergman. 2004. Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* **43**:1–20.

Nakahara, H., S. Amari-Si, and O. Hikosaka. 2002. Self-organization in the basal ganglia with modulation of reinforcement signals. *Neural Comput.* **14**:819–844.

Nakahara, H., H. Itoh, R. Kawagoe, Y. Takikawa, and O. Hikosaka. 2004. Dopamine neurons can represent context-dependent prediction error. *Neuron* **41**:269–280.

Nicola, S.M., D.J. Surmeier, and R.C. Malenka. 2000. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Ann. Rev. Neurosci.* **23**:185–215.

Partridge, J.G., S. Apparsundaram, G.A. Gerhardt, J. Ronesi, and D.M. Lovinger. 2002. Nicotinic acetylcholine receptors interact with dopamine in induction of striatal long-term depression. *J. Neurosci*. **22**:2541–2549.

Ravel, S., E. Legallet, and P. Apicella. 2003. Responses of tonically active neurons in the monkey striatum discriminate between motivationally opposing stimuli. *J. Neurosci.* **23**:8489–8497.

Ravel, S., P. Sardo, E. Legallet, and P. Apicella. 2001. Reward unpredictability inside and outside of a task context as a determinant of the responses of tonically active neurons in the monkey striatum. *J. Neurosci.* **21**:5730–5739.

Reynolds, J.N., B.I. Hyland, and J.R. Wickens. 2001. A cellular mechanism of reward-related learning. *Nature* **413**:67–70.

Reynolds, J.N., and J.R. Wickens. 2000. Substantia nigra dopamine regulates synaptic plasticity and membrane potential fluctuations in the rat neostriatum, *in vivo*. *Neuroscience* **99**:199–203.

Satoh, T., S. Nakai, T. Sato, and M. Kimura. 2003. Correlated coding of motivation and outcome of decision by dopamine neurons. *J. Neurosci.* **23**:9913–9923.

Schultz, W., P. Dayan, and P.R. Montague. 1997. A neural substrate of prediction and reward. *Science* **275**:1593–1599.

Schultz, W., L. Tremblay, and J.R. Hollerman. 2003. Changes in behavior-related neuronal activity in the striatum during learning. *TINS* **26**:321–328.

Suri, R.E., and W. Schultz. 2001. Temporal difference model reproduces anticipatory neural activity. *Neural Comput*. **13**:841–862.

Sutton, R.S., and A.G. Barto. 1998. Reinforcement Learning: An Introduction. Cambridge, MA: MIT Press.

Suzuki, T., M. Miura, K. Nishimura, and T. Aosaki. 2001. Dopamine-dependent synaptic plasticity in the striatal cholinergic interneurons. *J. Neurosci.* **21**:6492–6501.

Thut, G., W. Schultz, U. Roelcke et al. 1997. Activation of the human brain by monetary reward. *Neuroreport* **8**:1225–1228.

Ungless, M.A., P.J. Magill, and J.P. Bolam. 2004. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science* **303**:2040–2042.

Wickens, J.R., A.J. Begg, and G.W. Arbuthnott. 1996. Dopamine reverses the depression of rat corticostriatal synapses which normally follows high-frequency stimulation of cortex *in vitro*. *Neuroscience* **70**:1–5.

Wickens, J.R., J.N. Reynolds, and B.I. Hyland. 2003. Neural mechanisms of reward-related motor learning. *Curr. Opin. Neurobiol.* **13**:685–690.

Wilson, C.J., H.T. Chang, and S.T. Kitai. 1983. Origins of post synaptic potentials evoked in spiny neostriatal projection neurons by thalamic stimulation in the rat. *Exp. Brain Res*. **51**:217–226.

Yamada, H., N. Matsumoto, and M. Kimura. 2004. Tonically active neurons in the primate caudate nucleus and putamen differentially encode instructed motivational outcomes of action. *J. Neurosci.* **24**:3500–3510.

From left to right: Paul Bolam, Jim Surmeier, Ann Graybiel, Jeff Wickens,
Hagai Bergman, Dietmar Plenz, and Minoru Kimura (Sebastian Seung not pictured)

9

Group Report: Microcircuits, Molecules, and Motivated Behavior

Microcircuits in the Striatum

J. P. BOLAM, Rapporteur

H. BERGMAN, A. M. GRAYBIEL, M. KIMURA, D. PLENZ, H. S. SEUNG, D. J. SURMEIER, and J. R. WICKENS

ABSTRACT

The aim of this chapter is to bring together data from anatomical, neurochemical, physiological, and behavioral studies in an attempt to understand how the properties of the microcircuits of the striatum can underlie behavior in reward-related paradigms. The canonical microcircuit of the striatum in relation to corticostriatal and dopaminergic afferents is first described. Mechanisms of the selection of "appropriate assemblies of cortical neurons" for the required behavior, by the microcircuit and through the action of the neuromodulators, dopamine and acetylcholine, on corticostriatal synapses are described. The roles of dopaminergic afferents to the striatum and cholinergic neurons within the striatum in reward-related paradigm are discussed. Finally, a mechanism of how the microcircuit, at both the cellular and molecular level, can interface with global brain function in the production of reward-related behavior is discussed.

INTRODUCTION

The essential organization of the microcircuits of the striatum consists of a massive, topographic and heterogeneous input from the whole of the cortical mantle onto the striatum. This projection imparts functionality onto the striatum, which is essentially maintained throughout the cortex–basal ganglia thalamo–cortical loops. Unlike the cortex, the striatum consists of a single layer of neurons, the medium spiny neurons, which are the main targets of the afferent input to the basal ganglia, including that from the cortex, and are the output neurons of the

striatum. Information coded in striatal neurons seems to be different from that in the cortex: the response properties of close neighbors are not similar.

From a functional viewpoint, it is clear that the striatum, and indeed the basal ganglia in general, are involved in a variety of behaviors. For instance, striatal neurons in monkeys fire during memory-guided saccade paradigms (Hikosaka et al. 1989), and similarly, global activation occurs in the rat striatum during T-maze learning (Jog et al. 1999). However, a broad spatial preference of a saccade-related striatal neuron shows a marked modulation of spatial preference when reward is introduced into the paradigm. Similarly, once rats in the T-maze have learned the location of the reward, striatal neurons fire at the beginning of the maze, that is, in expectation of the reward, and in response to goal-reaching at the end of the maze. Thus, the properties of circuits in the striatum show remarkable plasticity with respect to behavioral context, reward, and learning.

A common feature of these behaviors is the *motivation* to act. These aspects of behavior and the plasticity are dependent on the dopamine input to the striatum from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). Thus, the striatum takes sensory, motor, cognitive, and limbic signals from the cortex; the dopamine input, which is carrying motivational or salience information (and reward-expectation error), modifies and shapes the response of the spiny neurons to these cortical signals, which then leads to the context-dependent behavior (Figure 9.1).

These observations imply a selection mechanism; we propose that the function of striatal microcircuits, as part of the cortex–basal ganglia–cortex loop, that are modified by the dopamine input, is to disregard unfavorable outcomes in favor of those that produce the reward. In this chapter, we discuss how the microcircuits of the striatum operate in this "selection" process by addressing four issues:

1. The fundamental or canonical microcircuit.
2. How "selection" can operate in this microcircuit.
3. Plasticity of this microcircuit, particularly in relation to the roles of dopamine (DA) and acetylcholine (ACh).
4. Plasticity of this microcircuit in relation to reward-related behaviors.

THE CANONICAL MICROCIRCUIT

In light of currently available anatomical and electrophysiological data, we propose that the striatal canonical microcircuit consists of two medium spiny projection neurons (MSN), one fast-spiking (FS) GABAergic interneuron, and one cholinergic neuron (Figure 9.2). The MSNs are GABAergic and receive excitatory glutamatergic inputs at the heads of their spines. An individual MSN possesses about 15,000 spines, and it is estimated that about half of these receive input from cortical terminals (Table 9.1). MSNs are also recipients of other

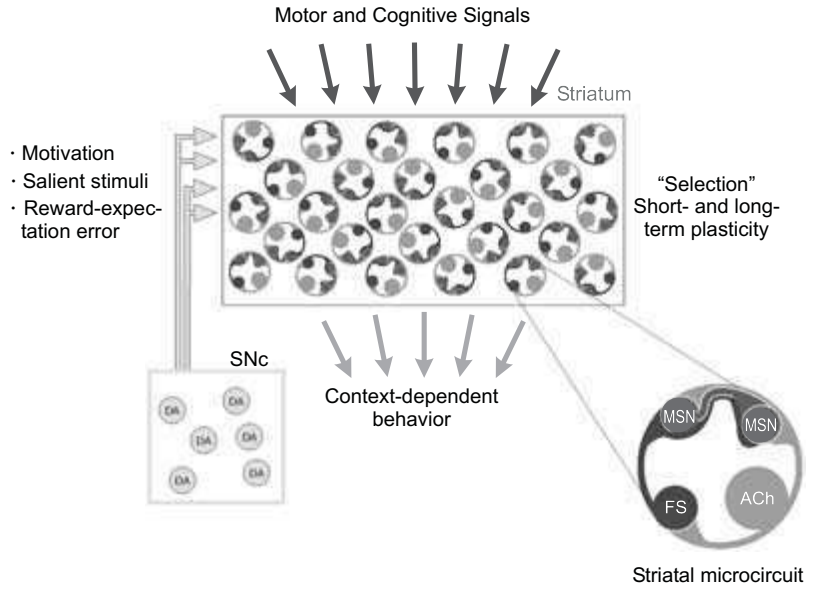

Figure 9.1 Diagrammatic representation of the functional organization of the striatum. The striatum receives motor and cognitive signals primarily from the cortex; the dopamine (DA) input, which is carrying motivational or salience information (and reward-expectation error), together with the striatal microcircuit, modifies and shapes the response of the spiny neurons to the cortical input, which then leads to the context-dependent behavior. For details of the striatal microcircuit see Figure 9.2.

afferent inputs to the striatum including the thalamus (to heads of spines and dendritic shafts; not considered further here) and, importantly for this discussion, dopaminergic input from ventral midbrain (mainly to spine necks and dendritic shafts). They also receive input from striatal interneurons, two of which are critical for the present discussion: the FS interneurons (mainly to the perikarya and proximal dendrites) and the cholinergic interneurons (similar distribution of terminals as the DA input). MSNs themselves give rise to local axon collaterals, the main targets of which are the dendritic shafts of other MSNs. The local axonal arbor is largely co-extensive with the dendritic arbor (average diameter 250 μm). MSNs give rise to the output of the striatum, one population projects to the external globus pallidus (GPe) and one projects to the output nuclei of the basal ganglia, the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr) but also providing collaterals to the GPe, and a third population (not considered in detail here) projects to the SNc.

FS GABAergic interneurons account for only a small proportion of striatal neurons, are mainly of medium size, and, like many other fast-spiking interneurons, express the calcium-binding protein, parvalbumin (PV). The main excitatory input to these neurons is also from the cortex, although they do receive

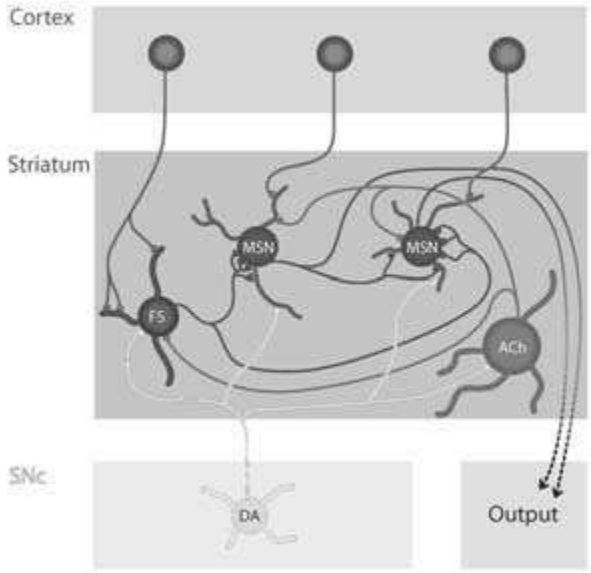

Figure 9.2 The canonical microcircuit of the striatum consists of two medium spiny projection neurons (MSN), one fast-spiking (FS) GABAergic interneuron, and one cholinergic interneuron (ACh). The MSNs are the major output neurons of the striatum and are synaptically interconnected (usually at the level of the dendritic shafts) and receive input from the other two classes of neurons: the FS GABAergic interneuron (at the level of perikarya and proximal dendrites) and ACh interneurons (on perikarya and dendrites). The ACh interneurons also innervate the FS GABAergic interneurons. Critical to the operation of the microcircuit are two afferents of the striatum: first, the corticostriatal projection that innervates the spines of MSNs and the dendrites of FS GABAergic interneurons (as well as cholinergic neurons, not illustrated), and second, the dopaminergic input from the ventral midbrain (DA) that innervates all classes of neurons in the canonical microcircuit.

input from the GPe as well as from the thalamus and cholinergic interneurons. These cells give rise to a dense axonal arbor that is again largely co-extensive with the dendritic arbor (average diameter about 250 μm**)**. The main output of FS neurons is to the perikarya and proximal dendrites of the MSN. There are a large number of PV-positive terminals innervating the proximal regions of MSNs, and it has been estimated that 4–27 PV cells converge onto an individual MSN (Koós and Tepper 1999). Furthermore, FS neurons are in a position to influence the activity of large numbers of MSNs, as their axonal arbors possess in the region of 5000 synaptic boutons, and they have been estimated to contact 135–541 MSNs (Koós and Tepper 1999).

The cholinergic neurons are the "giant" aspiny neurons of the striatum, having a dendritic diameter of up to one millimeter. They receive their afferent innervation from the thalamus, the cortex, DA terminals, and from MSNs. Their

Table 9.1 Quantitative aspects of synapses in the striatal microcircuit.

Presynaptic cell type	Total number of presynaptic cells	Number of synapses per presynaptic cell	Number of synapses per postsynaptic MSN
Corticostriatal pyramidal cell	380,000 in dendritic volume of one spiny neuron (Zheng & Wilson 2002)	40 in dendritic volume of one spiny neuron (Zheng & Wilson 2002)	7,500[a] (Zheng & Wilson 2002)
Medium spiny neuron (MSN)	2,791,000 (Oorschot 1996)	592[b] (Lee et al. 1997)	592[b] (Lee et al. 1997)
Cholinergic interneuron	12,200 (Oorschot 1997)	220,000 [c]	636[c]
GABA/PV interneuron	16,900 (Luk & Sadikot 2001)	5,000	30
Dopamine neuron	7,200 (Oorschot 1996)	370,000[d]	954[d]

[a] Assumes 50% asymmetric synapses are corticostriatal (Groves et al. 1994).
[b] Based on mean number of varicosities per striatonigral neuron (749) times fraction of varicosities with synaptophysin (0.79) (Lee et al. 1997).
[c] Assumes 6% of symmetric synapses are cholinergic (Groves et al. 1994).
[d] Assumes 9% of symmetric synapses are dopaminergic (Groves et al. 1994).

massive axonal arbor, which is far more extensive than that of the other classes, gives rise to many thousands of terminals and, possibly, even hundreds of thousands. One of the main targets of these terminals are MSNs, innervating spine necks, dendritic shafts, and perikarya.

Quantitative aspects of the cellular constituents of the striatal microcircuit provide some clues about the connectivity among them. There are an estimated 2,700,000 medium-sized spiny projection neurons (Oorschot 1996) and 16,900 aspiny GABA/PV interneurons (Luk and Sadikot 2001) in the rat striatum. Current estimates suggest each spiny projection neuron makes in the order of 600 synaptic contacts within the striatum (Oorschot et al. 2002; Wickens 2002). Estimates of the probability of connections based on realistic values of synapse density, extent of axonal and dendritic spread, and the volume of the region of overlap suggest that a pair of spiny projection neurons situated 100 μm apart, with axonal and dendritic arborizations extending up to 200 μm from the soma, would have a low probability of a synaptic contact from one cell to the other (Oorschot et al. 2002; Wickens 2002). This low probability of connections suggests that each spiny neuron probably makes synaptic contact with several

hundred others. Experimental data suggest that the average number of synapses between MSNs is 2.9 (Koós et al. 2004). Because of the low number of synapses and their dendritic location, the connection involves smaller synaptic currents and a higher failure rate than that of the FS neurons (Tunstall et al. 2002; Koós et al. 2004; Tepper and Plenz, this volume).

The FS/GABA interneurons (PV-positive) make many more synaptic contacts per neuron than the MSNs (mean of 7; Koós et al. 2004). There are an estimated 5000 boutons per interneuron. On the other hand, these interneurons constitute less than 1% of the total population of striatal neurons in the rat, and the ratio of spiny neuron to GABA interneuron is on the order of 165:1. Thus, based on these estimates, 5% of the inhibitory synaptic input to a spiny cell is from GABA interneurons and 95% from other spiny cells. These figures do not take into account extrinsic sources of GABA synapses, for example, from GP.

To gain an understanding of the functionality of the circuit, it is important to consider the quantitative aspects of the cortical inputs to both MSNs and FS neurons. It has been estimated (Kincaid et al. 1998) that, within the volume of striatum occupied by a single MSN, there are in the region of 380,000 cortical axons. Based on inter-bouton distances of filled corticostriatal axons, an individual axon gives rise to a maximum of 40 synapses in the same volume of striatum. Since there are about 2840 spiny neurons overlapping in the same volume, a single axon can then only contact less than 1.4% of MSNs. Thus, striatal spiny neurons with overlapping dendritic volumes have few cortical axons in common and cortical axons have few MSNs in common. This implies that individual spiny neurons will receive a massive convergence of individual cortical axons innervating their 15,000 spines and close neighbors will have a dramatically different complement of cortical afferents. There is thus a low degree of anatomical convergence (at the single cortical cell level) but a high degree of convergence of cortical neurons from a particular cortical region. It should be noted, however, that experimental data suggests that the pattern of cortical innervation of the PV-positive neurons (FS GABA interneurons) is different from that of MSNs, in that individual cortical axons frequently form multiple synaptic contacts with an individual PV-positive neuron (Ramanathan et al. 2002). This difference may account for the fact that FS GABA interneurons are more easily activated following cortical stimulation.

Quantitative aspects of the connectivity of the components of the "canonical microcircuit" are summarized in Table 9.1.

It is important to note that we have pared down the microcircuit to those elements that we believe are critical in the selecting and shaping of cortical inputs to the MSN. We have omitted several of the other well-established afferents of the striatum. At this stage, we also consider only those MSNs innervating basal ganglia output nuclei (GPi and SNr) and do not consider in the microcircuit the heterogeneous architecture of the striatum (striosomes and matrix as well as matrisomes located within the maxtrix.

HOW CAN "SELECTION" OPERATE IN THIS MICROCIRCUIT?

Leaving the dopaminergic and cholinergic neurons aside for the moment, the three neurons together (i.e., two MSNs and one FS neuron) form at least one feedback circuit (MSN to MSN connection) and one feedforward circuit (FS neuron to MSN). Based on the morphology, connectivity, and physiology of this microcircuit, we propose three basic methods for the "selection" of corticostriatal inputs:

1. Convergence of large numbers of cortical terminals onto an individual MSN.
2. Lateral inhibition between MSNs.
3. Feedforward inhibition of MSNs mediated by FS interneurons.

Convergence of Cortical Terminals onto an Individual Neuron

As indicated above, an individual MSN receives convergent input from about 7500 corticostriatal terminals, which probably reflects close to the same number of cortical pyramidal neurons. Given the highly hyperpolarized resting potential of MSNs (–80 mV) and the relatively small size of EPSP initiated by a single excitatory input, many corticostriatal inputs must converge almost simultaneously onto a spiny projection neuron to bring it into the sufficiently depolarized state—the UP state (see below)—to enable action potentials to be initiated. The precise number of simultaneous inputs or inputs occurring in a narrow time window to bring the neurons to the UP state is controversial and estimates vary from tens to thousands.

UP/DOWN States in Spiny Projection Neurons

Action potential firing of MSN in awake animals typically occurs in brief episodes separated by longer periods of relative quiescence (Kimura et al. 1990; Schultz and Romo 1988; Jog et al. 1999). These patterns of firing have also been demonstrated in intracellular records made from MSNs in immobilized, locally anaesthetized rats (Wilson and Groves 1981) and in urethane-anaesthetized rats (Wilson 1993). Intracellular recordings of MSNs in intact animals reveals large amplitude membrane potential fluctuations from a hyperpolarized DOWN state to a depolarized UP state (Wilson and Groves 1981; Wilson and Kawaguchi 1996; Wickens and Wilson 1998). These UP state transitions are brought about by the synaptic input from the cortex and probably also the thalamus. Thus UP state transitions do not occur after removal or deactivation of the cortex (Wilson 1993) and do not occur *in vitro* in the absence of excitatory drive, as in the brain slice at rest. On the other hand, UP states can be readily elicited in spiny projection neurons with just a minimal requirement of excitatory inputs. For example,

in cortex–striatum slice co-cultures, the spontaneous activity of the cortical culture is sufficient to drive striatal neurons into UP states (Plenz and Aertsen 1996; Plenz and Kitai 1998). Similarly, UP/DOWN state-like transitions occur in striatal slices in which at least part of the corticostriatal pathway is maintained and both cortex and striatum are exposed to excitatory agents, for example, NMDA (Vergara et al. 2003). These *in vivo* and *in vitro* results demonstrate that UP and DOWN state transitions reflect a network response of the striatum to synchronized excitatory inputs. This network response might be facilitated *in vivo* during sleep and local or general anesthesia, which are known to enhance cortical synchrony. Besides direct excitatory or generally depolarizing synaptic inputs, active properties of the dendritic membrane could contribute to UP state transitions and seem to be required to maintain the UP state under certain conditions (see below). Of the many ion channels expressed by MSNs (Table 9.2), actions on specific channels have been identified that control the generation and maintenance of the UP state in MSNs, including:

- Initiation of the UP state transition is dependent upon the interaction between inwardly rectifying (Kir2) K^+ channels and the excitatory synaptic input arising from the cortex/thalamus.
- The transition to the UP state is controlled in its initial phases by rapidly activating voltage-dependent conductances as the Kir2 channels close. The most prominent of these channels are carried by Na^+ (Nav1.1,1.6) and K^+ (Kv1.2, Kv4).

With maintained depolarization, other K^+ channels (KCNQ) enter the picture to help limit the extent of depolarization. In addition, there is evidence that other depolarizing inward conductances (Cav1.3 Ca^{2+}, Na^+) play a role in some circumstances to generate dendritic plateau potentials that help to maintain the UP state. It is important to note that these conductances are under the influence of neuromodulators and, at least in the case of Cav1.3 channels, are positioned within spines where glutamatergic inputs driving the UP state transition are placed. Thus, this is a potential key site for the control of plasticity in the striatum (see below).

Although all MSNs show UP state transitions, not all fire action potentials spontaneously (Stern et al. 1998). Action potential firing, when it occurs, happens only in the UP state. UP state transitions, however, do not necessarily lead to action potential firing and occur in silent as well as spontaneously firing cells (Wilson and Groves 1981). Thus the convergence of many cortical afferents sufficiently depolarizes the membrane to a level at which specific channels come into operation (see below) to maintain it at that level. From this UP state, then, subsequent excitatory inputs will lead to the initiation of action potentials. Therefore, the UP state is necessary, but not sufficient, for action potential firing.

In addition to the large amplitude shifts in membrane potential that occur with UP state transitions, numerous small amplitude, noise-like, fluctuations in

Table 9.2 Electrophysiologically characterized currents in spiny projection neurons.

Electrophysiologically defined current	**References**
I_{Kir} (inwardly rectifying Kir2 K^+ channel)	Hagiwara and Takahashi (1974) Leech and Stanfield (1981) Mermelstein et al. (1998) Nisenbaum and Wilson (1995) Uchimura et al. (1989)
I_{As} (slowly inactivating Kv1 K^+ channel)	Gabel and Nisenbaum (1998) Nisenbaum et al. (1998, 1994) Nisenbaum and Wilson (1995) Shen et al. (2004) Surmeier et al. (1991, 1992)
I_{Na} (Na^+ Nav1.1, 1.2, 1.6 channel)	Cepeda et al. (1995) Chao and Alzheimer (1995) Fraser et al. (1993) Hoehn et al. (1993) Ogata and Tatebayashi (1990) Schiffmann et al. (1995) Surmeier et al. (1992) Surmeier and Kitai (1997)
L (Cav1.2, Cav1.3 L-type channel)	Bargas et al. (1991, 1994) Hernandez-Lopez et al. (2000)
N, P, R (Cav 2.1, 2.2, 2.3)	Bargas et al. (1994) Mermelstein et al. (1999) Surmeier et al. (1995)
I_{Krp} (persistent KCNQ K^+ channel)	Nisenbaum et al. (1996) Shen et al. (2004)
I_{Af} (rapidly inactivating A-type, Kv4 K^+ channel)	Surmeier et al. (1988, 1989) Tkatch et al. (2000)

membrane potential appear superimposed on the UP and DOWN states. In spontaneously firing neurons, these noise-like fluctuations in membrane potential trigger action potential generation. Similar fluctuations are also observed in silent MSNs but they do not reach threshold for action potential firing, although this can occur if the membrane potential is brought closer to threshold by injection of depolarizing current (Wilson and Kawaguchi 1996).

In dual *in vivo* intracellular recordings from anaesthetized animals, transitions between UP and DOWN states are highly correlated (Stern et al. 1998). However, during a synchronized UP state, action potential firing is not synchronized. The large amplitude transitions may represent the firing of assemblies of cortical cells, while the small amplitude fluctuations represent the fine temporal structure of activity within an active assembly.

Thus, the UP state can be seen as a selection process that will enable spiny projection neurons to control basal ganglia output, but will not necessarily guarantee participation in this control.

Lateral Inhibition between Medium Spiny Projection Neurons

The classical view of the organization of the striatum is that the GABAergic lateral interaction between MSNs generates a "winner-take-all" network through mutual suppression of action potential generation at the soma. Recordings from pairs of connected neurons suggest that this is *not* the case and that the interaction can take many forms.

Current available data (see Tepper and Plenz, this volume) suggest that the GABAergic synapse between spiny projection neurons is not significantly different from other GABAergic synapses described in, for example, the cortex. Although an individual MSN receives a large number of terminals from other MSNs, because of the low probability of connections, the connections between an individual pair will be sparse. The synapse reveals a large variability in probability of release and displays short-term plasticity that is under control of neuromodulators. So far, short-term facilitation has been reported, which suggests that the transmission supports action potential bursts (Czubayko and Plenz 2002). However, short-term depression and modulation of short-term plasticity by DA has also been demonstrated (Koós et al. 2004). Taken together, these data imply that synaptic transmission between spiny projection neurons is highly variable and contributes to the temporal (short-term plasticity) and spatial (local axon collateral) dynamics of the striatal network.

The IPSC is significantly weaker at the soma than the IPSC originating from the FS interneuron (Koós et al. 2004; Tepper et al. 2004). However, the many nonlinear aspects of MSN electrophysiology provide for a rich repertoire on which this synapse could operate. First, the positive chloride reversal potential with respect to the DOWN state could allow this synapse to depolarize MSNs, thereby changing intrinsic ion channel states. Second, the predominant location of the synapse on dendrites suggests participation in the control of dendritic rather than somatic processing. For example, instead of being involved in suppressing action potential generation at the soma, MSN input to other MSN dendrites could control the temporal relationship between synaptic input and backpropagating action potentials (see below), terminate/start dendritic plateau potentials, or delay/advance action potential firing in the postsynaptic neurons.

These possible effects deviate from the classical idea that GABAergic transmission between spiny projection neurons generates a "winner-take-all" network through mutual suppression of action potential generation at the soma. It is thus clearly necessary that we characterize the nature of interaction between MSNs, not least because these synapses represent a high proportion of the

inhibitory, or rather, GABAergic, input to these cells. One possible function of the collateral synapse is discussed below.

Spike Backpropagation in MSNs (see Figure 9.3)

The strong plasticity in the corticostriatal pathway (see below) raises the question whether there is a mechanism that allows those synapses that participate in the generation of an output signal to be regulated specifically. This problem, commonly known as "credit assignment," has been suggested to be solved by a nonlinear/supralinear interaction between activated NMDA receptors in the dendrite and a backpropagating action potential into the dendrite. In this context, the NMDA receptor with bound glutamate at the active synapse provides the "flag" for which the synapse participates in an input to the neuron, whereas the backpropagation of the somatic action potentials signals back to the input that an output has been generated. The specificity occurs at the level of the intracellular calcium dynamics. In a largely simplified scheme, the backpropagating spike releases the magnesium block from NMDA receptors and those receptors that have glutamate bound allow calcium to enter the cell. This signal will control the regulation of synaptic plasticity at that synapse.

Is spike backpropagation in MSNs an important element that controls plasticity in the corticostriatal pathway? This question can be broken down into several different aspects of the problem. Experiments have been performed *in vitro* in mature cortex–striatum–substantia nigra organotypic co-cultures. In this *in vitro* system, striatal UP and DOWN states are highly comparable with UP and DOWN state fluctuations *in vivo* under urethane anesthesia with respect to, for example, spontaneous firing during UP states, UP state durations, and delay to first action potential distributions in the UP state. These similarities suggest a similarly balanced excitatory drive in the *in vitro* system compared to *in vivo*.

The results of these analyses demonstrate the following:

1. Intracellular calcium signals are not saturated in MSN dendrites despite large UP and DOWN state membrane potential fluctuations of $\sim$40 mV. Instead, the action potential number during UP states is precisely encoded in the calcium transient peak for all dendritic compartments.
2. During the DOWN state, action potentials from somatic current injections trigger strong calcium transients in higher-order dendrites. The fact that calcium transients disappear when voltage-gated sodium channels are blocked in dendrites, strongly suggests that spike backpropagation occurs in MSNs.
3. During the UP state, somatic spikes also trigger calcium transients in higher-order dendrites of MSNs. This is an important point because the UP state changes the electrotonic properties of MSNs dramatically, and it was previously questionable whether spike backpropagation could operate under these circumstances.

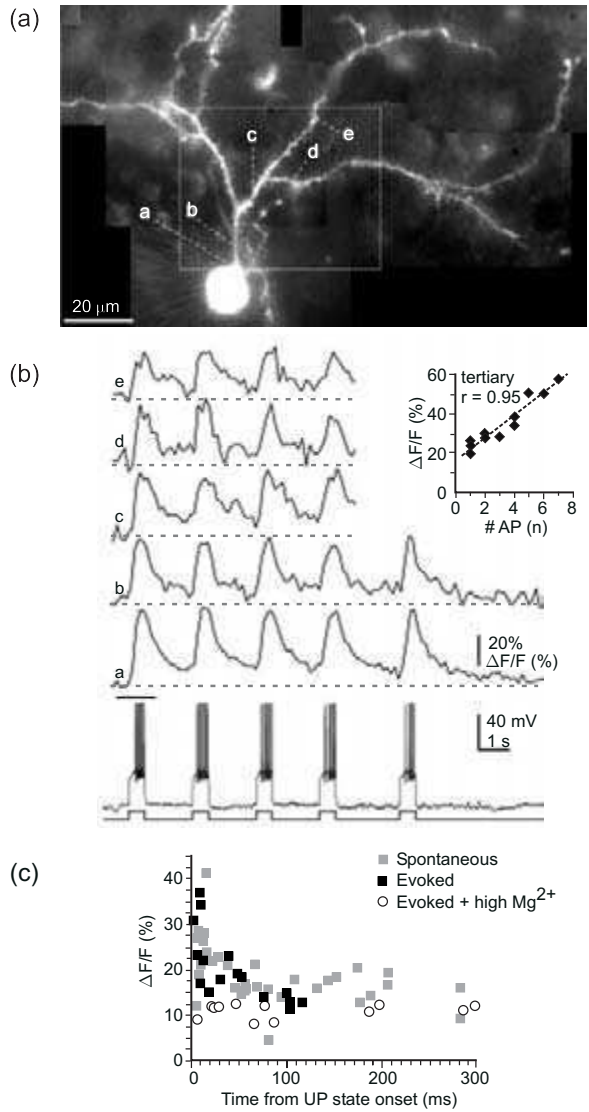

Figure 9.3 Spike backpropagation in MSNs. Through spike backpropagation, dendritic calcium transients encode the action potential (AP) bursts and action potential timing in spiny projection neurons. (a) Spiny projection neuron filled with calcium indicator Fura-2 (cortex–striatum–substantia nigra organotypic culture). (b) Repetitive action potential bursts by somatic current injection (bottom trace) elicit reliably calcium transients in soma, primary, and higher-order dendrites. The amplitude of the calcium transient is correlated linearly with the number of spontaneous somatic spikes during UP states (inset; Kerr and Plenz 2002). Characters correspond to locations in (a). (c) The dendritic calcium signal (tertiary dendrite) encodes the timing of the first single spike with respect to UP state onset through an NMDA-dependent mechanism (Kerr and Plenz 2004).

4. Calcium transients from somatic action potentials were supralinear in higher-order dendrites and could be blocked by intracellular blockade of the NMDA receptor.
5. The supralinear transients have a clear time-dependency with respect to UP state transition and spike timing. The earlier the neurons fire a somatic spike after a transition into an UP state, the stronger the resulting supralinear calcium transient in higher-order dendrites through spike backpropagation.

Taken together, all three elements that are necessary to solve the "credit assignment" problem have thus been demonstrated in MSNs in the organotypic slice preparations. First, somatic spikes backpropagate into spiny projection neuron dendrites during the UP state. Second, they interact with active synapses via the NMDA receptor. Third, this interaction is supralinear and crucial for the "credit assignment" problem.

These data open a new avenue in the control of corticostriatal input processing between spiny projection neurons. Inhibitory inputs from other spiny projection neurons by controlling the timing of spike backpropagation into the dendrite of the postsynaptic neuron might control corticostriatal plasticity in these neurons (see also below). The question that remains to be answered is whether this situation is found in MSNs *in vivo*. Differences in the synaptic environment of the organotypic culture from that found *in vivo* may alter dendritic channel and spine densities in such a way as to reduce the probability that somatic spikes are faithfully backpropagated. The advent of 2 photon laser scanning microscopy will allow resolution of this question in the near future.

Feedforward Inhibition of MSNs Mediated by FS Neurons

FS GABA interneurons innervate the proximal regions of MSNs (although the connection is not reciprocal) and an individual FS neuron will innervate probably in the region of several hundred MSNs. Paired recordings of the connections between FS neurons and MSNs reveals that FS neurons provide a strong, reliable inhibition of MSNs (see Tepper and Plenz, this volume).

Although we do not know the precise function of this inhibitory connection, it may shunt or block the cortically driven action potentials. In doing this, one of the actions may be to erase a previous constellation of spiking/nonspiking MSNs and through this may facilitate a new network configuration/selection for the future.

In light of the predominantly perisomatic targeting of the GABAergic input from FS neurons to MSNs, one of their main actions might be the timing of somatic action potentials. Because of the intense innervation of a given striatal volume by many FS interneurons, the input of FS neurons might be interpreted as setting a timing window for when spiking is allowed to occur in MSNs. In that sense, FS neurons provide a temporal framework that guides action potential

generation in the striatal microcircuit. This temporal framework could be enhanced by several mechanisms. For instance, synchronization between FS neurons might provide a spatially uniform framework that, through oscillations within the interneuron network, might set up a rhythmic framework for spiking in MSNs. Similarly, afferent control of the FS interneuron network through cortical afferents (excitatory) or inhibitory afferents (from GPe) could be interpreted as setting up unique or repetitive timing frameworks. Thus, FS interneurons operate to "select" a population of MSNs in both a spatial and temporal framework (Courtemanche et al. 2003; Parsatharathy and Graybiel 1997).

PLASTICITY OF THE MICROCIRCUIT PARTICULARLY IN RELATION TO THE ROLES OF DOPAMINE AND ACETYLCHOLINE

Plasticity of the corticostriatal and thalamostriatal pathways, brought about by reward-related input from DA neurons in the SNc and VTA, is a probable basis for the learning-related changes in striatal responses measured in single-unit studies in behaving animals. Such changes in the activity of the output neurons of the striatum may lead to changes in the probability of responses. If the rules for induction and maintenance of synaptic changes are appropriate, the resulting changes in synaptic efficacy may lead to an increased probability of behavioral responses that lead to rewards and produce an overall maximization of accumulated rewards.

The detailed requirements for induction of synaptic plasticity in the corticostriatal pathway are gradually being elucidated, but much remains to be determined before these requirements can be described with mathematical precision. Current findings support the hypothesis of a three-factor rule for synaptic modification in the striatum, in which presynaptic activity, postsynaptic depolarization, and neuromodulator activity may play a role. Each of these factors has temporal and magnitude characteristics that may influence the extent and direction of changes in synaptic efficacy.

It is well established that long-term depression (LTD) can be induced in the corticostriatal synapses by high-frequency stimulation (HFS) of the cerebral cortex. LTD is a depolarization-dependent process that requires activation of L-type calcium channels in the postsynaptic cell during the conditioning HFS and an increase in intracellular calcium concentration (Bonsi et al. 2003; Lovinger and Tyler 1996). These conditions are likely to be met in striatal cells that fire action potentials in response to excitatory synaptic input (Kerr and Plenz 2002).

Long-term potentiation (LTP) has also been reported in the striatum. Initial reports of striatal LTP were based on the effects of HFS in slices bathed in magnesium-free medium (Calabresi et al. 1992). Both DA depletion and the dopamine D_1 receptor antagonists block LTP in magnesium-free conditions (Kerr

and Wickens 2001). Dopamine, applied in a manner that mimics the natural release of DA produced by reward, is sufficient to facilitate LTP (Wickens et al. 1996). In these latter experiments, DA was applied in brief pulses coinciding with a pre- and postsynaptic conjunction of activity. The pulsatile application of DA reversed the LTD, which normally follows HFS, and potentiation of responses was induced.

Experiments *in vivo* using electrical stimulation of DA neurons in the substantia nigra have shown that endogenous release of DA evoked by behaviorally reinforcing stimulation parameters induces a potentiation of corticostriatal synapses (Reynolds et al. 2001). In addition, the degree of potentiation up to 10 min after the stimulus trains was correlated with the rate of learning of intracranial self-stimulation behavior.

Preliminary studies have indicated precise temporal requirements for the DA-dependent induction of LTP. If DA pulses that induce LTP when applied simultaneously with pre- and postsynaptic conjunction of activity are delayed by as little as 500 ms, LTD occurs instead. This strict temporal requirement argues against a cellular eligibility trace corresponding to the delay of reinforcement gradient measured behaviorally. The effectiveness of delayed reinforcers may depend on the ability of the DA system to produce a reward-prediction error in advance of the actions that lead to reward (Figure 9.4).

Plasticity of the Corticostriatal Synapse at the Molecular Level

Dopamine receptors interact with ion channels in a variety of ways (Table 9.3). One molecular mechanism proposed to underlie plasticity at the corticostriatal synapse at the level of the spine relates to modulation of the activity of L-type Ca^{2+} channels (Surmeier, this volume; Cepeda et al. 1998; Liu et al. 2004). These channels are placed strategically to a large degree in dendritic spines close to the site of glutamatergic corticostriatal and DA synapses. Activation of the D_1 type of DA receptor prolongs the UP state and increases excitability of MSNs, whereas activation of D_2 receptors reduces UP state and decreases excitability. This effect is mediated in part through phosphorylation/dephosphorylation, respectively, of L-type Ca^{2+} channels, increasing and reducing the availability of Ca^{2+} respectively. Furthermore, ACh release from the cholinergic interneurons depresses excitability at the level of the spine (but not the somatodendritic tree) by an action through M_1 muscarinic receptors and dephosphorylation of the channel. Thus, the classical view of a reciprocal relationship between DA and ACh in the striatum is reflected at the single channel level in spines. The interaction between DA and ACh is regulated by a small phosphoprotein referred to as "regulator of calmodulin signaling" (RCS). When phosphorylated by protein kinase A (PKA), RCS increases dramatically its affinity for Ca^{2+}/calmodulin, effectively blocking Ca^{2+} signaling. When D_1 receptor stimulation leads to the activation of PKA and phosphorylation of RCS, M_1 receptor suppression of

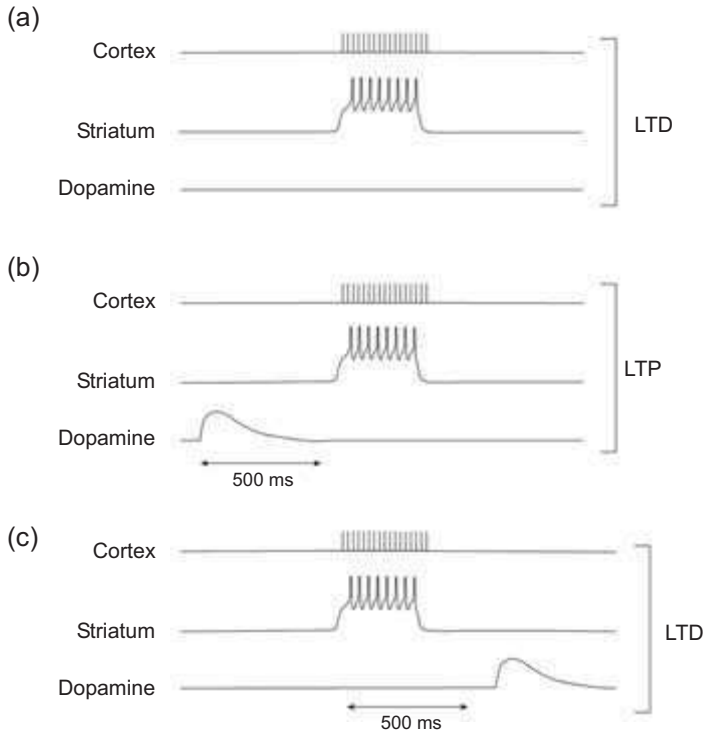

Figure 9.4 Effect of dopamine on activity-dependent synaptic plasticity in the corticostriatal pathway. (a) A conjunction of cortical presynaptic activity and striatal postsynaptic activity leads to long-term depression (LTD) in the absence of a dopamine pulse. (b) A conjunction of cortical presynaptic activity and striatal postsynaptic activity leads to long-term potentiation (LTP) if preceded by a dopamine pulse. (c) The same conjunction of cortical presynaptic activity and striatal postsynaptic activity leads to LTD if the DA pulse is delayed.

L-type Ca^{2+} channels in spines is effectively blocked. Thus, the timing of DA and ACh signals has important consequences for the regulation of Ca^{2+} dynamics in spines and possibly synaptic plasticity.

It is clear that D_1 receptor stimulation promotes excitatory events and UP-state generation in *striatonigral* MSNs. The question arises as to whether there is a corresponding signaling pathway in *striatopallidal* MSNs, that is, those neurons that predominantly express D_2 receptors. (Gerfen et al. 1995; Surmeier et al. 1996). This population of MSNs also expresses adenosine A_{2a} receptors. These receptors have the same biochemical linkages as D_1 receptors. The electrophysiological effects have not yet been investigated, but there is the potential that the cortical glutamatergic signal may be translated by 5′-nucleotidase into a "teaching" signal to these neurons, corresponding in some way to the dopaminergic signal in the D_1-expressing neurons.

Table 9.3 Dopamine receptor subtype-specific effects on ion channels.

Channel	Dopamine D_1 receptor activation	Dopamine D_2 receptor activation
I_{Kir}	Increased (Galarraga et al. 1994; Pacheco-Cano et al. 1996)	Increased* (Freedman and Weight 1988, 1989) or decreased (Uchimura and North 1990)
I_{As}	Decreased (Surmeier and Kitai 1997)	Increased (Surmeier and Kitai 1997)
I_{Na}	Reduced (Surmeier et al. 1992)	Reduced increase by D_2 (Surmeier et al. 1992)
L	Increased (Hernandez-Lopez et al. 1997; Surmeier et al. 1995)	Decreased (Hernandez-Lopez et al. 2000)
N, P	Decreased (Surmeier et al. 1995)	Decreased Surmeier et al. (1995)

* This modulation is likely to be of a Kir3 channel in a novel type of striatal neuron.

PLASTICITY OF STRIATAL MICROCIRCUITS AND REWARD-RELATED BEHAVIORS

The DA neurons of the ventral midbrain, which provide a massive and widespread innervation of the striatum (Table 9.1), respond with a brief increase or decrease in rate of firing to both the onset of *reward-predicting stimuli* and *reinforcers* as an outcome of action (decision) in classical conditioning task (Schultz et al. 1997; Fiorillo et al. 2003) or a voluntary decision task for reward (Satoh et al. 2003) (see Figure 9.5). Responses to positive and negative reinforcers precisely encode reward-expectation error. Responses to reward-predicting stimuli depend on the probability of reward. In an instrumental conditioning task with voluntary decision for reward, however, they might not encode levels of reward expectation but rather the levels of motivation, because the magnitude of responses is negatively correlated with behavioral reaction time (Action 1 in Figure 9.5). On the other hand, at the same reward-expectation level, coding reward-expectation error is positively correlated with coding motivation level (Satoh et al. 2003). This means that coding reward-expectation error by the firing rate of DA neurons is significantly modulated by motivation in such a way that gain of error coding is high when the level of motivation is high. Thus, the response of an animal to important environmental stimuli, especially a reward, is a brief increase in their firing rate and hence an increased release of DA in the striatum. Omission of expected reward (Schultz et al. 1997) and aversive stimuli (Ungless et al. 2004) induce a brief suppression in firing.

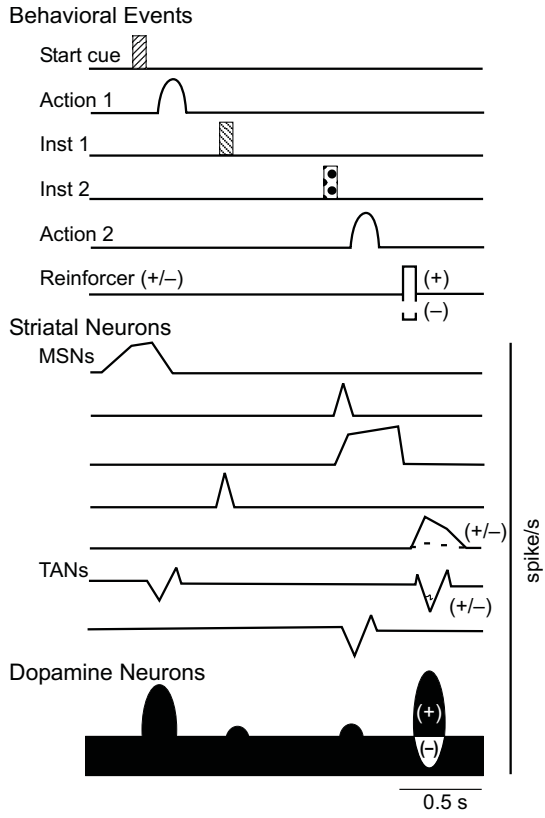

Figure 9.5 Schematic illustration of behavioral events and striatal and dopamine neuron activity during behavioral decision task for a reward in monkey. The task was initiated by illumination of the start LED on the push button as a first reward-predicting stimulus. The monkeys depressed the illuminated start button. The start LED was turned off 400 ms after the monkeys continued to hold the button. Then, the target LEDs (Inst 1) and a GO LED (Inst 2) were simultaneously activated. The monkeys were required to continue depressing the start button for variable lengths of time before the GO LED was turned off. They released the start button and depressed one of the three illuminated target buttons. If an incorrect button was depressed, a beep sound with a low tone occurred, and the next trial began by illuminating the start LED. Because the monkey remembered the incorrect button selected at the first trial, it made a choice between the two remaining buttons. If the monkey made an incorrect choice again, the third trial started after a low-tone beep and the monkey depressed the remaining, single correct button. If the correct button was depressed, a beep sound with a high tone occurred, and a small amount of reward water was delivered through the spout attached to the monkey's mouth. The high-tone and low-tone beep sounds served as positive and negative reinforcers, respectively, after the behavioral decisions. Once the monkeys found the correct button, the same button was used as the correct button in the succeeding trials. Various types of medium-size spiny neurons (MSNs) and cholinergic interneurons (TANs) show characteristic activity in relation to the behavioral events, while midbrain dopamine neurons respond to the start LED and reinforcer beep as a single group of neurons.

A population of neurons in the striatum is tonically active (TANs) and has been correlated with the large cholinergic interneurons, the fourth neuron in our canonical microcircuit. Recordings from these neurons in similar paradigms, as used above for the study of DA neurons (Yamada et al. 2004) or, indeed, simultaneous recordings of both DA neurons and TANs (Morris et al. 2004), have revealed that TANs respond selectively to sensory stimuli instructing *outcomes* of action, such as reward, aversive stimuli like air puff to an animal's face, or a sound instructing no-reward (Blazquez et al. 2002). They respond selectively to sensory stimuli instructing the motivational outcomes of action, stimuli for trigger action, and reinforcers. Reward is not necessarily special for TANs. Different groups of TANs respond more readily to reward than aversive stimuli, whereas other groups of TANs prefer aversive stimuli, and still other groups prefer a sound instructing no-reward. Thus, TANs encode the salience instructed motivational contexts, i.e., they report that an important event is about to occur (Blazquez et al. 2002). TANs in the putamen and caudate nucleus represent similar, but quantitatively different, aspects of information. The temporal response pattern of TANs in these paradigms is more or less stereotypical. There is a brief pause of tonic firing for 100 to 150 ms, followed by an increase above baseline firing. In some instances, the pause responses are preceded by a brief increase in firing.

Thus, DA neurons and TANs code events related to motivational outcomes. Additionally, DA neurons encode reward-expectation error precisely. TANs can discriminate different kinds of motivational outcomes. Therefore, information encoded by DA cells and cholinergic interneurons can work as teaching signals in different ways. The response of TANs warns the striatum about a salient event, it tells the striatum to listen. The response of DA neurons is to tell the striatum about the reinforcement.

MSNs in the striatum of awake monkeys discharge at a very low mean rate, usually less than 1 spike/s. Although their discharge properties in behavioral tasks depend on the location in the striatum, which relates to the topography of inputs from the cerebral cortex, it is common for nearby MSNs to show quite different properties, such as reward-related activity, limb or eye movement-related activity, and responses to sensory instructions. This is because of the large-scale convergence of different types of information of cortical origin onto single MSNs and the wide-scale divergence between neighboring MSNs (see above). In the behavioral task shown in Figure 9.5, MSNs are activated in relation to various aspects of task requirements. The activity of some groups of neurons increases gradually up to the start cue and Action 1. Another group of neurons show phasic activation after Instruction 1 (Inst) or Instruction 2. Still another group of neurons show burst discharges in relation to Action 2 or reinforcers. Thus, the different types of MSNs participate in encoding aspects of task requirements in a discrete manner. An important property of these different types of MSN activity is dependence on motivational state; that is, most MSNs show

stronger activation when rewarding outcomes are expected (Kawagoe et al. 1998; Lauwereyns et al. 2002; Cromwell and Schultz 2003), whereas smaller number of neurons are activated more strongly when smaller or no-reward outcome is expected (Watanabe et al. 2002).

MICROCIRCUITS, MOLECULES, AND BEHAVIOR

We have now identified a potential canonical microcircuit in the striatum, several possible ways in which the microcircuit itself can select which populations of MSNs fire and the manner in which they fire, ways in which corticostriatal synapses are plastic, and changes in the activity of DA-containung neurons in the ventral midbrain and cholinergic neurons in the striatum during specific behaviors. How can these be brought together to understand how a microcircuit can interface with global brain function in the production of behavior?

From the foregoing discussion, it is clear that one of the roles of DA in the striatum is to mediate/facilitate synaptic plasticity. Dopamine D_1 receptor stimulation is critical in the expression of LTP in the corticostriatal pathway; DA can strengthen corticostriatal synapses on the MSNs. Thus, one action of the brief increase in DA release at spines in response to reward-predicting stimuli may be to potentiate selectively the striatal synaptic inputs from the assembly of cortical neurons that are required to express the appropriate behavior. Thus striatal activity and the DA teaching signal allow the microcircuit to potentiate selectively the response to the assembly of cortical neurons for the appropriate behavior.

What then might be the role of the simultaneous depression of ACh that occurs with the increased release of DA? As indicated above, one mechanism of increasing excitability may be to influence the availability/levels of calcium through an action on the L-type Ca^{2+} channel located in spines. The decrease in ACh release and the increase in DA release would have similar net effects on the channel leading to increased probability of opening and hence increased excitability of excitatory cortical synapses. Thus the two modulators acting in concert would be in a position to provide a selective potentiation of the synapses of the "appropriate assembly of cortical neurons" related to the behavior.

Lateral interaction between MSNs will further sculpt the response of groups of MSNs to the selected group of cortical afferents and the feedforward inhibition through the FS interneurons will further "select," in both spatial and temporal domains, the group of MSNs that will fire. What the "appropriate assembly of striatal neurons" is that matches the appropriate assembly of cortical neurons is critically influenced by the divergent–convergent anatomy of corticostriatal projections (Graybiel et al. 1994). The striatum has modules, about the size of cortical columns, that organize its inputs and outputs. Striatal interneurons are also differentially represented in these compartments (called striosomes and matrisomes), and the DA–ACh modulation of striatal activity is also compartmentally selective. It thus seems likely that this architecture strongly influences

the function of striatal microcircuits. The increased or altered pattern of activity of the selected group of MSNs will then lead to the "appropriate" behavior via the basal ganglia output nuclei and their connections to subcortical premotor regions or connections with frontal cortical regions via the thalamic.

The scenario we describe here leaves many questions unanswered and raises many new questions relating to the functional organization of the striatum and the basal ganglia in general. Nevertheless, the microcircuit that we have described brings together data from anatomical, neurochemical, physiological, and behavioral studies and provides a rational basis for future studies of the basal ganglia.

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REFERENCES

Bargas, J., A. Howe, J. Eberwine, Y. Cao, and D.J. Surmeier. 1994. Cellular and molecular characterization of Ca^{2+} currents in acutely isolated, adult rat neostriatal neurons. *J. Neurosci*. **14**:6667–6686.

Bargas, J., D.J. Surmeier, and S.T. Kitai. 1991. High- and low-voltage activated calcium currents are expressed by neurons cultured from embryonic rat neostriatum. *Brain Res*. **541**:70–74.

Blazquez, P., N. Fujii, J. Kojima, and A.M. Graybiel. 2002. A network representation of response probability in the striatum. *Neuron* **33**:973–982.

Bonsi, P., A. Pisani, G. Bernardi, and P. Calabresi. 2003. Stimulus frequency, calcium levels, and striatal synaptic plasticity. *Neuroreport* **14**:419–422.

Calabresi, P., A. Pisani, N.B. Mercuri, and G. Bernardi. 1992. Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of NMDA receptor channels. *Eur. J. Neurosci.* **4:**929–935.

Cepeda, C., S.H. Chandler, L.W. Shumate, and M.S. Levine. 1995. Persistent Na^{+} conductance in medium-sized neostriatal neurons: Characterization using infrared videomicroscopy and whole-cell patch-clamp recordings. *J. Neurophysiol.* **74**:1343–1348.

Cepeda, C., C.S. Colwell, J.N. Itri, S.H. Chandler, and M.S. Levine. 1998. Dopaminergic modulation of NMDA-induced whole-cell currents in neostriatal neurons in slices: Contribution of calcium conductances. *J. Neurophysiol.* **79**:82–94.

Chao, T.I., and C. Alzheimer. 1995. Do neurons from rat neostriatum express both a TTX-sensitive and a TTX-insensitive slow Na^+ channel? *J. Neurophysiol*. **74**: 934–941.

Courtemanche, R., N. Fujii, and A.M. Graybiel. 2003. Synchronous, focally modulated β-band oscillations characterize local field potential activity in the striatum of awake behaving monkeys. *J. Neurosci.* **23**: 11,741–11,752.

Cromwell, H.C., and W. Schultz. 2003. Effects of expectations for different reward magnitudes on neuronal activity in primate striatum. *J. Neurophysiol*. **89**:2823–2838.

Czubayko, U., and D. Plenz. 2002. Fast synaptic transmission between striatal spiny projection neurons. *PNAS* **99**:15,764–15,769.

Fiorillo, C.D., P.N. Tobler, and W. Schultz. 2003. Discrete coding of reward probability and uncertainty by dopamine neurons. *Science* **299**:1898–1902.

Fraser, D.D., K. Hoehn, S. Weiss, and B.A. MacVicar. 1993. Arachidonic acid inhibits sodium currents and synaptic transmission in cultured striatal neurons. *Neuron* **11**:633–644.

Freedman, J.E., and F.F. Weight. 1988. Single K^+ channels activated by D_2 dopamine receptors in acutely dissociated neurons from rat corpus striatum. *PNAS* **85**:3618–3622.

Freedman, J.E., and F.F. Weight. 1989. Quinine potently blocks single K^+ channels activated by dopamine D_2 receptors in rat corpus striatum neurons. *Eur. J. Pharmacol*. **164**:341–346.

Gabel, L.A., and E.S. Nisenbaum. 1998. Biophysical characterization and functional consequences of a slowly-inactivating potassium current in neostriatal neurons. *J. Neurophysiol.* **79**:1989–2002.

Galarraga, E., M.T. Pacheco-Cano, J.V. Flores-Hernandez, and J. Bargas. 1994. Subthreshold rectification in neostriatal spiny projection neurons. *Exp. Brain Res*. **100**:239–249.

Gerfen, C.R., K.A. Keefe, and E.B. Gauda. 1995. D_1 and D_2 dopamine receptor function in the striatum: Coactivation of D_1 and D_2 dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D_1-containing neurons. *J. Neurosci*. **15**:8167–8176.

Graybiel, A.M., T. Aosaki, A.W. Flaherty, and M. Kimura. 1994. The basal ganglia and adaptive motor control. *Science* **265**:1826–1831.

Groves, P.M., J.C. Linder, and S.J. Young. 1994. 5-hydroxydopamine-labeled dopaminergic axons: Three-dimensional reconstructions of axons, synapses, and postsynaptic targets in rat neostriatum. *Neuroscience* **58**:593–604.

Hagiwara, S., and K. Takahashi. 1974. The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. Membr. Biol*. **18**:61–80.

Hernandez-Lopez, S., J. Bargas, D.J. Surmeier, A. Reyes, and E. Galarraga. 1997. D_1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca^{2+} conductance. *J. Neurosci*. **17**:3334–3342.

Hernandez-Lopez, S., T. Tkatch, E. Perez-Garci et al. 2000. D_2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca^{2+} currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. *J. Neurosci.* **20**:8987–8995.

Hoehn, K., T.W. Watson, and B.A. MacVicar. 1993. A novel tetrodotoxin-insensitive, slow sodium current in striatal and hippocampal neurons. *Neuron* **10**:543–552.

Hikosaka, O., M. Sakamoto, and S. Usui. 1989. Functional properties of monkey caudate neurons I. Activities related to saccadic eye movements. *J. Neurophysiol*. **61**: 780–798.

Jog, M.S, Y. Kubota, C.I. Connolly, V. Hillegaart, and A.M. Graybiel. 1999. Building neural representations of habits. *Science* **286**:1745–1749.

Kawagoe, R., Y. Takikawa, and O. Hikosaka. 1998. Expectation of reward modulates cognitive signals in the basal ganglia. *Nat. Neurosci*. **1**:411–416.

Kerr, J.N.D., and D. Plenz. 2002. Dendritic calcium encodes striatal neuron output during UP-states. *J. Neurosci.* **22**:1499–1512.

Kerr, J.N.D., and D. Plenz. 2004. Action potential timing determines dendritic calcium during striatal UP-states. *J. Neurosci*. **24**:877–885.

Kerr, J.N.D., and J.R. Wickens. 2001. Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum *in vitro*. *J. Neurophysiol.* **85**: 117–124.

Kimura, M., M. Kato, and H. Shimazaki. 1990. Physiological properties of projection neurons in the monkey striatum to the globus pallidus. *Exp. Brain Res.* **82**:672–676.

Kincaid, A.E., T. Zheng, and C.J. Wilson. 1998. Connectivity and convergence of single corticostriatal axons. *J. Neurosci*. **18**:4722–4731.

Koós, T., and J.M. Tepper. 1999. Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nat. Neurosci*. **2**:467–472.

Koós, T., J.M. Tepper and C.J. Wilson. 2004. Comparison of IPSCs evoked by spiny and fast-spiking neurons in the striatum. *J. Neurosci.* **24**:7916–7922.

Lauwereyns, J., K. Watanabe, B. Coe, and O. Hikosaka. 2002. A neural correlate of response bias in monkey caudate nucleus. *Nature* **418**:413–417.

Lee, T., T. Kaneko, R. Shigemoto, S. Nomura, and N. Mizuno. 1997. Collateral projections from striatonigral neurons to substance P receptor-expressing intrinsic neurons in the striatum of the rat. *J. Comp. Neurol*. **388**:250–264.

Leech, C.A., and P.R. Stanfield. 1981. Inward rectification in frog skeletal muscle fibres and its dependence on membrane potential and external potassium. *J. Physiol*. **319**:295–309.

Liu, J.C., R.A. DeFazio, A. Espinosa-Jeffrey et al. 2004. Calcium modulates dopamine potentiation of N-methyl-D-aspartate responses: Electrophysiological and imaging evidence. *J. Neurosci. Res.* **76**:315–322.

Lovinger, D.M., and E. Tyler. 1996. Synaptic transmission and modulation in the neostriatum. *Intl. Rev. Neurobiol.* **39**:77–111.

Luk, K.C., and A.F. Sadikot. 2001. GABA promotes survival but not proliferation of parvalbumin-immunoreactive neurons in rodent neostriatum: An *in vivo* study with stereology. *Neuroscience* **104**:93–103.

Mermelstein, P.G., R.C. Foehring, T. Tkatch et al. 1999. Properties of Q-type calcium channels in neostriatal and cortical neurons are correlated with beta subunit expression. *J. Neurosci.* **19**:7268–7277.

Mermelstein, P. G., W.J. Song, T. Tkatch, Z. Yan, and D.J. Surmeier. 1998. Inwardly rectifying potassium (IRK) currents are correlated with IRK subunit expression in rat nucleus accumbens medium spiny neurons. *J. Neurosci.* **18**:6650–6661.

Morris, G., D. Arkadir, A. Nevet, E. Vaadia, and H. Bergman. 2004. Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* **43**:133–143.

Nisenbaum, E.S., P.G. Mermelstein, C.J. Wilson, and D.J. Surmeier. 1998. Selective blockade of a slowly inactivating potassium current in striatal neurons by (+/–) 6-chloro-APB hydrobromide (SKF82958). *Synapse* **29**:213–224.

Nisenbaum, E.S., and C.J. Wilson. 1995. Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. *J. Neurosci*. **15**: 4449–4463.

Nisenbaum, E.S., C.J. Wilson, R.C. Foehring, and D.J. Surmeier. 1996. Isolation and characterization of a persistent potassium current in neostriatal neurons. *J. Neurophysiol*. **76**:1180–1194.

Nisenbaum, E.S., Z.C. Xu, and C.J. Wilson. 1994. Contribution of a slowly inactivating potassium current to the transition to firing of neostriatal spiny projection neurons. *J. Neurophysiol.* **71**:1174–1189.

Ogata, N., and H. Tatebayashi. 1990. Sodium current kinetics in freshly isolated neostriatal neurones of the adult guinea pig. *Pflugers Arch*. **416**:594–603.

Oorschot, D.E. 1996. Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: A stereological study using the Cavalieri and optical dissector methods. *J. Comp. Neurol.* **366**:580–599.

Oorschot, D.E. 1997. Total number of large interneurons within the rat neostriatum: A stereological study using the optical disector and Cavalieri methods. *Intl. J. Neurosci.* **89**:90.

Oorschot, D.E., M.J. Tunstall, and J.R. Wickens. 2002. Local connectivity between striatal spiny projection neurons: A re-evaluation. In: The Basal Ganglia VII, ed. L. Nicholson and R.L.M. Faull, pp. 421–434. New York: Plenum.

Parthasarathy, H.B., and A.M. Graybiel. 1997. Cortically driven immediate-early gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. *J. Neurosci.* **17**:2477–2491.

Pacheco-Cano, M.T., J. Bargas, S. Hernandez-Lopez, D. Tapia, and E. Galarraga. 1996. Inhibitory action of dopamine involves a subthreshold Cs(+)-sensitive conductance in neostriatal neurons. *Exp. Brain Res*. **110**:205–211.

Plenz, D., and A. Aertsen. 1996. Neural dynamics in cortex–striatum co-cultures. II. Spatio-temporal characteristics of neuronal activity. *Neuroscience* **70**:893–924.

Plenz, D., and S.T. Kitai. 1998. "Up" and "down" states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in striatal fast-spiking interneurons studied in cortex-striatum-substantia nigra organotypic cultures. *J. Neurosci.* **18**:266–283.

Ramanathan, S., J.J. Hanley, J.-M. Deniau, and J.P. Bolam. 2002. Synaptic convergence of motor and somatosensory cortical afferents onto GABAergic interneurons in the rat striatum. *J. Neurosci*. **22**:8158–8169.

Reynolds, J.N.J., B.I. Hyland, and J.R. Wickens. 2001. A cellular mechanism of reward-related learning. *Nature* **413**:67–70.

Satoh, T., S. Nakai, T. Sato, and M. Kimura. 2003. Correlated coding of motivation and outcome of decision by dopamine neurons. *J. Neurosci*. **23**:9913–9923.

Schiffmann, S.N., P.-M. Lledo, and J.D. Vincent. 1995. Dopamine D1 receptor modulates the voltage-gated sodium current in rat striatal neurones through a protein kinase A. *J. Physiol.* **483**:95–107.

Schultz, W., P. Dayan, and P.R. Montague. 1997. A neural substrate of prediction and reward. *Science* **275**:1593–1599.

Schultz, W., and R. Romo. 1988. Neuronal activity in the monkey striatum during the initiation of movements. *Exp. Brain Res.* **71**:431–436.

Shen, W., S. Hernandez-Lopez, T. Tkatch, J.E. Held, and D.J. Surmeier. 2004. Kv1.2-containing K+ channels regulate subthreshold excitability of striatal medium spiny neurons. *J. Neurophysiol*. **91**:1337–1349.

Stern, E.A., D. Jaeger, and C.J. Wilson. 1998. Membrane potential synchrony of simultaneously recorded striatal spiny neurons *in vivo*. *Nature* **394**:475–478.

Surmeier, D.J., J. Bargas, H.C. Hemmings, A.C. Nairn, and P. Greengard. 1995. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* **14**:385–397.

Surmeier, D.J., J. Bargas, and S.T. Kitai. 1988. Voltage-clamp analysis of a transient potassium current in rat neostriatal neurons. *Brain Res*. **473**:187–192.

Surmeier, D.J., J. Bargas, and S. Kitai. 1989. Two types of A-current differing in voltage-dependence are expressed by neurons of the rat neostriatum. *Neurosci. Lett.* **103**:331–337.

Surmeier, D.J., J. Eberwine, C.J. Wilson et al. 1992. Dopamine receptor subtypes colocalize in rat striatonigral neurons. *PNAS* **89**:10,178–10,182.

Surmeier, D.J., and S.T. Kitai. 1997. State-dependent regulation of neuronal excitability by dopamine. *Nihon Shinkei Seishin Yakurigaku Zasshi* **17**:105–110.

Surmeier, D.J., W.J. Song, and Z. Yan. 1996. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.* **16**:6579–6591.

Surmeier, D.J., A. Stefani, R.C. Foehring, and S.T. Kitai. 1991. Developmental regulation of a slowly-inactivating potassium conductance in rat neostriatal neurons. *Neurosci. Lett*. **122**:41–46.

Surmeier, D.J., Z.C. Xu, C.J. Wilson, A. Stefani, and S.T. Kitai. 1992. Grafted neostriatal neurons express a late-developing transient potassium current. *Neuroscience* **48**: 849–856.

Tepper, J.M., T. Koós, and C.J. Wilson. 2004. GABAergic microcircuits in the neostriatum. *TINS* **27**:662–669.

Tkatch, T., G. Baranauskas, and D.J. Surmeier. 2000. Kv4.2 mRNA abundance and A-type K(+) current amplitude are linearly related in basal ganglia and basal forebrain neurons. *J. Neurosci.* **20**:579–588.

Tunstall, M.J., D.E. Oorschot, A. Kean, and J.R. Wickens. 2002. Inhibitory interactions between spiny projection neurons in the rat striatum. *J. Neurophysiol*. **88**:1263–1269.

Uchimura, N., E. Cherubini, and R.A. North. 1989. Inward rectification in rat nucleus accumbens neurons. *J. Neurophysiol*. **62**:1280–1286.

Uchimura, N., and R.A. North. 1990. Actions of cocaine on rat nucleus accumbens neurones *in vitro*. *Brit. J. Pharmacol*. **99**:736–740.

Ungless, M.A., P.J. Magill, and J.P. Bolam. 2004. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science* **303**:2040–2042.

Vergara, R., C. Rick, S. Hernandez-Lopez et al. 2003. Spontaneous voltage oscillations in striatal projection neurons in a rat corticostriatal slice. *J. Physiol.* **553:**169–182.

Watanabe, K., J. Lauwereyns, and O. Hikosaka. 2002. Neural activity for reluctant saccades in monkey caudate nucleus. *Soc. Neurosci. Abstr.* **32**:280.

Wickens, J.R. 2002. Surround inhibition in the basal ganglia. In: The Basal Ganglia VI, ed. M. DeLong and A.M. Graybiel, pp. 187–197. New York: Plenum/Kluwer Press.

Wickens, J.R., A.J. Begg, and G.W. Arbuthnott. 1996. Dopamine reverses the depression of rat cortico-striatal synapses which normally follows high frequency stimulation of cortex *in vitro*. *Neuroscience* **70**:1–5.

Wickens, J.R., and C.J. Wilson. 1998. Regulation of action-potential firing in spiny neurons of the rat neostriatum *in vivo*. *J. Neurophysiol.* **79**:2358–2364.

Wilson, C.J. 1993. The generation of natural firing patterns in neostriatal neurons. *Prog. Brain Res.* **99**:277–297.

Wilson, C.J., and P.M. Groves. 1981. Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. *Brain Res.* **220**:67–80.

Wilson, C.J., and Y. Kawaguchi. 1996. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J. Neurosci.* **16**:2397–2410.

Yamada, H., N. Matsumoto, and M. Kimura. 2004. Tonically active neurons in the primate caudate nucleus and putamen differentially encode instructed motivational outcomes of action. *J. Neurosci*. **24**:3500–3510.

Zheng, T., and C.J. Wilson. 2002. Corticostriatal combinatorics: The implications of corticostriatal axonal arborizations. *J. Neurophysiol*. **18**:4722–4731.

10

Olfactory Microcircuits

Dynamics and Computation beyond the Receptor Neurons

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ABSTRACT

Recent results from the author's laboratory concerning the nature of odor-evoked patterns of responses of neurons and neural assemblies in the insect olfactory system are summarized. This summary is restricted to a description and functional interpretation of the transformations carried out in the antennal lobe and the mushroom body, the first two relay stations directly downstream of the large array of olfactory receptor neurons. The intent is to generate discussion about coding and computation in olfactory circuits, by placing the emphasis on the emergent properties of activity within local circuits.

1. Odor representations differ dramatically in the antennal lobe and mushroom body. Representations are distributed (across neurons and in time) in the antennal lobe, but are sparse, brief (1–2 spikes), and synthetic in the mushroom body, a format ideal for associative memories.
2. This difference results from active processing in the antennal lobe, which spreads out the representations provided by afferents and formats them for coincidence detection and synthesis in the mushroom body.
3. This process (*receptor activation → formatting → synthesis*) also provides possible mechanisms for tuning invariance, applicable to other sensory systems.
4. The phenomenological parallels with fish and mammalian olfactory systems suggest possible computational and functional similarities.
5. Olfactory networks seem ideal to explore and explain the roles of tuning curves, oscillatory synchronization, and sparseness in brain local circuits. Emphasis is given to the need to study neural activity as population patterns, circuit dynamics, and "receiver" properties as crucial steps towards deciphering neural codes.

INTRODUCTION

The last fifteen years have seen growing activity in the neuroscience of olfaction. The main impetus for this was the seminal discovery by Buck and

Axel of the first family of odorant receptor genes (Buck and Axel 1991; Buck 1996). This discovery finally allowed one to establish the dimensionality of odor representations across receptor arrays (the number of functionally different receptor types for one species now appears to vary between about 60 in *Drosophila melanogaster* and over a thousand in rodents or round worms) as well as the anatomical organization and "molecular logic" of early olfactory circuits (Mombaerts et al. 1996). Prior to this discovery, much electrophysiological work had been done to describe the responses of sensory and first-order (and to a lesser degree, second-order) neurons in the olfactory system of vertebrates (Kauer and Moulton 1974; Kauer 1987) and invertebrates (Hildebrand and Shepherd 1997). We now are able to combine the power and resolution of such physiological studies with the molecular identification and, increasingly, manipulation of the cellular components whose activity we monitor. Olfactory networks are thus ideal to study general issues of neural integration and computation at the scale of local circuits.

The focus of this Dahlem Workshop was on local neural circuits and function. The emphasis of this chapter has deliberately been placed on the emergent properties of activity within local circuits. While I have some faith in the relevance of traditional single-neuron studies in sensory neuroscience (e.g., tuning curves), I, like others before me (Singer and Gray 1995; Freeman 1978; Wilson and McNaughton 1993), believe that we run the risk of missing something important by ignoring population activity. By population activity I mean at least two things: (a) the co-relational features of neuronal activity, such as synchronization, and (b) the existence of patterns whose existence can be detected only when we simultaneously study multiple neurons. I argue that although activity should be monitored across neuronal assemblies, it should, whenever possible, be recorded with single-neuron and single-action potential resolution. The reason is simple: whereas we, as observers of neural activity, can focus our attention on one neuron at a time, the "decoders" or interpreters of the neural messages we study (i.e., the neurons directly postsynaptic to those we study) have a different perspective: they carry out some operation (that we will try to discover) on a complex pattern of synaptic input and fire action potentials when some global conditions are met. What the response probabilities of the individual presynaptic neurons are (i.e., to some extent, what their "tuning curves" are) is clearly important; but so is the correlation between activities in the neurons that share the same target.

This simple approach seems particularly relevant when studying olfactory coding: I will give two illustrative examples based on work in fish and in insects. In the zebrafish olfactory bulb, individual mitral cells generally respond to many odors, but with cell- and odor-specific patterns (Friedrich and Laurent 2001). Thus, if a mitral cell is tested with several odors (A–G), one obtains a group of response patterns that are more or less different from one another. If one is interested in describing the tuning curve of this mitral cell, one is faced with the

problem of defining the appropriate metric for a response. If, as observed experimentally, the mitral cell's responses are not stationary (i.e., if its responses to odors contain successive up- and down-modulations of firing frequency over several tens to hundreds of milliseconds), the mitral cell's firing rate should be measured over time epochs as long as the integration window of its postsynaptic targets (measuring mean firing rates over long time periods would be meaningless if the neuron's targets never get to integrate over such long epochs); that is, the targets' properties should define the analysis window. If the responses of a mitral cell to different odors are measured over relatively short time bins (100–200 ms), one finds that its tuning curve varies over time: it might fire best in response to odors D and G initially, but best to odors E and F 100 ms later (Friedrich and Laurent 2001). When seen in this light, one realizes that the traditional notions of tuning curve and odor preference are, at best, difficult to use with such neurons; at worst, they are inappropriate. What seems more relevant is to identify the assemblies of coactive mitral cells during each relevant time period; those assemblies ultimately determine the response probabilities of their targets. In other words, attention is shifted to the assembly of coactive neurons that share a target. Local circuit and connectivity thus define the method and scale of the analysis.

The second example illustrates the emergence of patterns from the study of neural assemblies. Locust projection neurons (the functional analog of vertebrate mitral cells) each respond to many odors (Perez-Orive et al. 2002). When a projection neuron is challenged with one odor at a variety of concentrations (e.g., over a 1000-fold range), one generally observes that its responses differ across concentrations. The projection neuron might respond strongly at low concentrations and be powerfully inhibited at high concentrations (Stopfer et al. 2003). As one progressively steps through the concentrations, one often observes that the projection neuron's responses change gradually over some concentration range, but abruptly transition to a completely new pattern at some arbitrary concentration. When a different projection neuron is tested with the same odor over the same concentration range, one observes the same general properties, but the trend might be opposite (i.e., PN2 might be inhibited at low concentration and excited at high) and the concentration at which the pattern inversion occurs might be different from that for PN1 (Stopfer et al. 2003). One can thus study tens to hundreds of projection neurons and describe tens to hundreds of such response patterns over concentrations and end up understanding very little about how concentration affects representations. However, when these responses are studied as patterns across the population (i.e., as activity vectors in a space defined by the neural population), one suddenly sees that all the population patterns evoked by the different concentrations of one odor are indeed continuously related to one another, and that they form families of representations that are all more closely related to each other than the families of representations of different concentrations of a different odor (Stopfer et al. 2003). In other

words, combining data from neuron populations (each recorded at single-cell and single-spike resolution) reveals an order not seen with single neurons. Below I argue that such analysis methods and scales are critical to understand olfactory coding. Each section summarizes a main point for discussion and is illustrated in a corresponding figure.

ODOR REPRESENTATIONS IN THE ANTENNAL LOBES AND MUSHROOM BODIES

Neural activity was recorded from neurons in the antennal lobes and mushroom bodies of locusts, honeybees, and fruitflies, using patch-clamp, intracellular, and extracellular electrodes. The antennal lobe is a glomerular structure, analogous to the vertebrate olfactory bulb. The mushroom body is a structure involved in the formation of odor memories (Menzel 1987). Its functional analog in vertebrates might be the piriform cortex, although the structural similarities between mushroom bodies and piriform cortex are much less obvious than those between antennal lobes and olfactory bulbs. The responses of the principal neurons of the antennal lobe (projection neurons, direct targets of the olfactory receptor neurons) and of the mushroom body (Kenyon cells, direct targets of the projection neurons) are compared here (Figure 10.1).

Odor representations are *distributed* and *dynamic* (on several timescales) in the antennal lobes but are *sparse, brief*, and *synthetic* in the mushroom bodies. Later figures will examine the mechanisms thought to underlie the transformation from the first to the second.

Neural Activity Is Distributed across the Population of Antennal Lobe Principal Neurons (Figure 10.1a)

These spike rasters represent the activity of 110 different projection neurons in the locust antennal lobe in response to the delivery of the same odor (hexanol, gray shadow). The projection neurons were recorded in identical conditions using tetrodes, in groups of about 10–20 projection neurons each, from different preparations. The locust antennal lobe contains about 830 projection neurons per antennal lobe. Note that many (about 50% on average) projection neurons respond to this odor, but that response patterns differ from projection neuron to projection neuron. Many responses are multiphasic and each response phase can last several hundred milliseconds. This type of response is typical in this system and suggests a distributed, dynamic representation. Similar patterns are observed in the antennal lobe of *Drosophila* (Wilson et al. 2004).

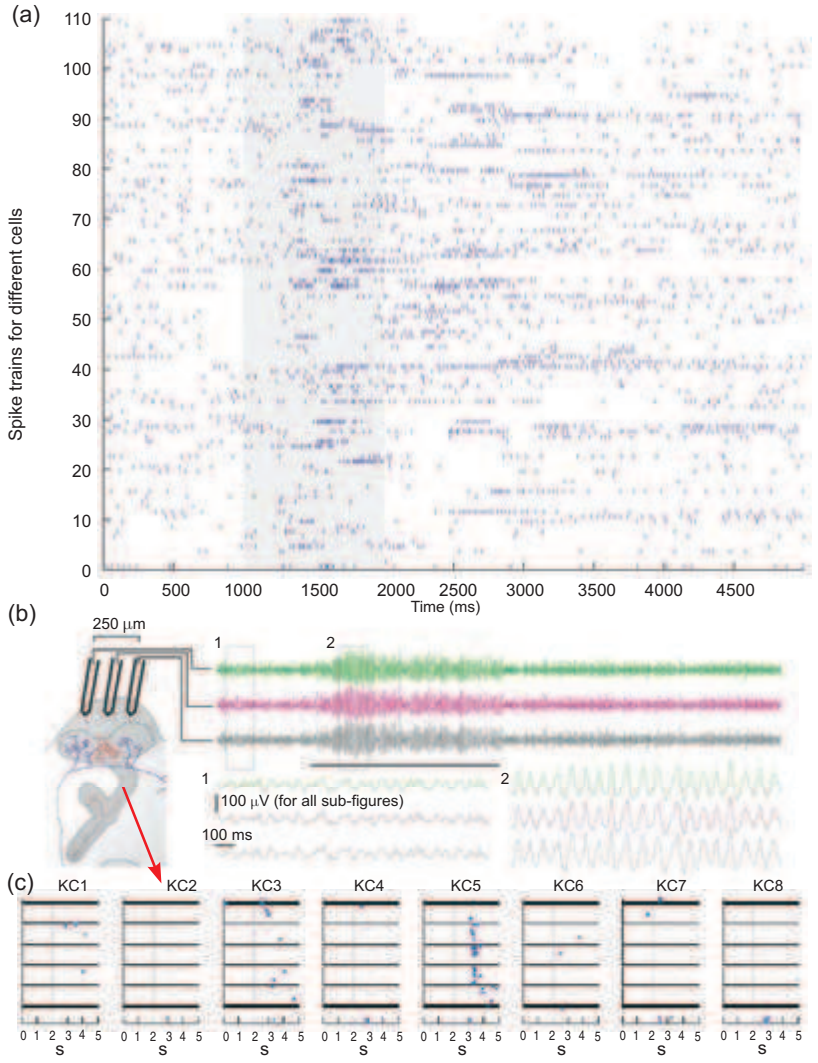

Figure 10.1 Odor representations are broadly distributed across antennal lobe (AL) projection neurons (PNs) and sparsely across mushroom body (MB) Kenyon cells (KCs). KCs are directly postsynaptic to PNs. Data recorded from locust AL PNs (a), MB calyx (b), and single MB neurons, KCs (c). (a) Responses of 110 PNs to the same odor (presented over the duration of the gray box). Note that many PNs respond, each with a particular firing pattern. Odor representations are thus distributed in space and time. (b) Simultaneous local field potential (LFP) recordings from 3 zones within the MB around (1) and during (2) a 10 s long odor pulse; note the high spatiotemporal correlation between the 3 distant recording sites at rest (a) or during the odor pulse (2). (c) Responses of 8 KCs to the same odor (100 ms pulses, gray bar) over 5 concentrations (rows, from top to bottom: 1, 0.1, 0.05, 0.01, 0.001; ten trials per concentration). Note the typically low response probability, short response duration, and occasional concentral invariance (KC5).

Projection Neurons Output to the Mushroom Body Is Characterized By Pairwise Transient Synchrony and Global Oscillations (Figure 10.1b)

Triple simultaneous local field potential recordings were made from the Kenyon cell soma cluster in the mushroom body, i.e., the target site of projection neurons, during a prolonged odor pulse (10 s, black horizontal bar). Note the occurrence of 20–30 Hz oscillations during the odor and the remarkable coherence across the three channels both at rest (box 1) and during the odor pulse (box 2). This spatial coherence and absence of phase delay is explained by the broad and lateral spread of all projection neurons' axonal collaterals in the mushroom body calyx (region where they contact Kenyon cell dendrites) and by the fact that these local field potentials (LFPs) reflect the dominant influence of projection neurons' collective synaptic output onto Kenyon cells. The patterns of projection neuron output in Figure 10.1a thus hide transient pairwise synchrony across projection neurons. As discussed below, each oscillation cycle can thus be characterized by a particular vector of projection neuron activity.

Kenyon Cell Responses Are Sparse, Highly Specific, and Often Concentration Invariant (Figure 10.1c)

Simultaneous tetrode recordings were made from eight Kenyon cells and responses of those Kenyon cells to ten trials of five concentrations of the same odor (Stopfer et al. 2003). Note that their background firing is extremely low (<0.05 spike/s on average) and that responses are rare and generally short (2.3 spike/s on average). Kenyon cell responses are highly specific and synthetic (i.e., encode feature combinations) (Perez-Orive et al. 2002).

THE ANTENNAL LOBE SPREADS ACTIVITY ACROSS THE PROJECTION NEURON POPULATION

Several hypotheses have been proposed for the role of the first olfactory relay (antennal lobe or olfactory bulb). One poses that it serves to sharpen the odor tuning of individual principal neurons, via lateral inhibition between like-tuned glomerular units (Mori and Shepherd 1994). Another suggests that it serves as a faithful relay of representations by olfactory afferent neurons (Wang et al. 2003). I propose that, with the exception of special odors (e.g., some pheromones), the antennal lobe spreads afferent activity across its component neurons to optimize the use of coding space. The system would act as a "mixing box." Information is not lost; rather it is distributed broadly across the population, in a step critical to the formation of synthetic representations by postsynaptic neurons (presented in Figures 10.4–10.7).

Early Olfactory Circuits in Insects (Figure 10.2a)

Olfactory sensory neurons at the base of sensilla converge on glomeruli where they contact projection neurons (uniglomerular in *Drosophila*, multiglomerular in locusts). Projection neurons then send an axon to protocerebral structures (mushroom bodies and/or lateral horn).

Projection Neurons in Glomerulus DM2 Are More Broadly Tuned than Their Afferents (Figure 10.2b)

Exploiting *Drosophila* genetics and olfactory receptor physiology by J. Carlson, it is possible to characterize the tuning of olfactory sensory neurons whose glomerular projections are known (Or22a here). Using patch-clamp recordings, the projection neurons projecting to DM2 are then sought and characterized under the same conditions. Both olfactory sensory neurons and projection neurons were tested with the same 34 odors at 10^{-3} dilution (Wilson et al. 2004). The pairs of histograms compare their tunings within two consecutive 100 ms long bins. Note that DM2 tuning of the projection neurons is considerably broader than that of their olfactory sensory neurons' input. If projection neurons are more broadly tuned than the receptor neurons to which they are presumably directly connected, since they share the same glomerulus, information must be distributed across the antennal lobe through local circuits.

The Redistribution of Activity across Projection Neurons Can Be Interpreted as Enabling a Better Use of Coding Space (Figure 10.2c)

A system of tightly tuned projection neurons that recapitulate or sharpen olfactory sensory neurons' tuning diminishes the chances of projection neuron coactivation and would thus concentrate coding to the volume around its main dimensions (that is, the glomerular preferences). A system of broadly tuned projection neurons, by contrast, spreads representations in the coding space defined by the projection neuron population. If average projection neuron response probability is close to 50%, as we observe, the use of projection neuron coding space is possibly optimal.

ODOR REPRESENTATIONS BY PROJECTION NEURONS CAN BE DESCRIBED AS FAMILIES OF TRAJECTORIES

Assume that the antennal lobe contains n projection neurons (or n groups of identically tuned projection neurons). These projection neurons define an n-dimensional coding space, in which odor representations can be thought of as

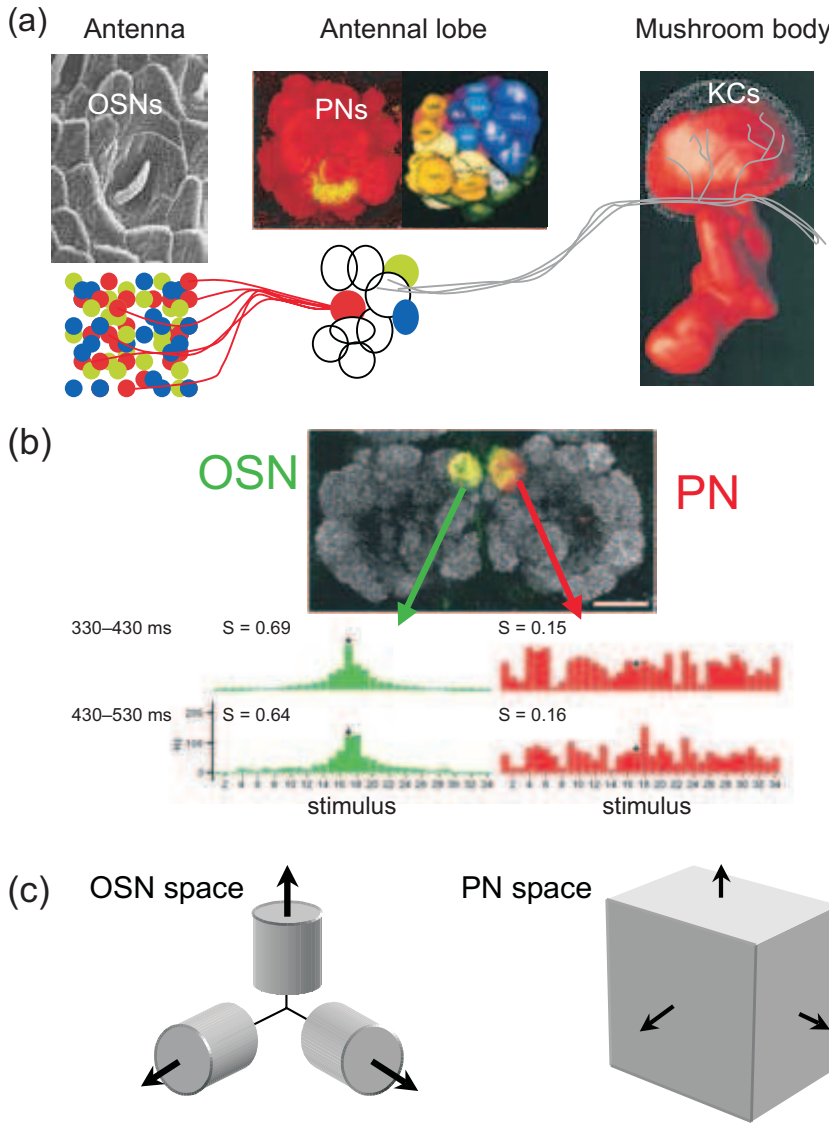

Figure 10.2 Odor-evoked activity spreads from olfactory receptor neurons to the projection neuron (PN) population, a transformation that is interpreted as leading to a better use of coding space. (a) Diagram of insect olfactory circuits. Olfactory sensory neurons (OSNs) of similar molecular make up converge from the antenna to the same glomeruli in the antennal lobe (Vosshall et al. 1999; 2000). There, OSN axons contact PNs and local neurons (not shown). PN axons then project to the lateral horn and, in most cases, to the mushroom body, a structure known for its role in associative memory. Kenyon cells (KCs) are the principal (and only intrinsic) neurons of the mushroom body and are directly postsynaptic to the antennal lobe PNs. *Caption continues on next page.*

vectors. These vectors are calculated using some measure of activity in each one of the *n* dimensions (e.g., mean firing rates of the *n* projection neurons). Because projection neuron responses are not stationary, however, mean rates are not appropriate. Rather, one measures firing rates in consecutive small time windows (where "small" means large enough to contain significant numbers of spikes, but smaller than the timescale over which the population pattern evolves), producing a sequence of representation vectors. These sequences of vectors in turn define trajectories. Each trajectory describes the evolution of the projection neuron population response over time, often described as a spatiotemporal pattern. This simple formalism is useful because it allows both a quantification of similarities between spatiotemporal patterns (e.g., across odors or concentrations), and a geometric visualization (Figure 10.3a) of these otherwise complex-looking (e.g., Figure 10.1a) activity patterns.

Families of Trajectories Defined during Long Odor Pulses (Figure 10.3a)

Before odor delivery, the population of projection neurons hovers around rest, due to noisy baseline activity (4–5 spike/s, largely uncorrelated across projection neurons). When the odor is delivered, specific sets of projection neurons are activated and push the system through a series of states (1, 2…), each of which correspond to an oscillation cycle (Figure 10.1a, right). When the odor stops, oscillatory synchronization ceases but projection neuron activity continues, defining the relaxation to the rest state. When several concentrations of the same odor are used (C_1, C_2…), they define a family of close trajectories (manifold) in the state space defined by the projection neurons (Figure 10.3a, left). Different odors define different manifolds (Stopfer et al. 2003).

Projection Neuron Population Behavior during Odor Plumes (Figure 10.3b)

Simultaneous recordings of LFP, antennal lobe neurons (here local neurons), and olfactory sensory neurons input (electroantennogram) in an odor plume

Figure 10.2 (*continued*) (b) Comparison of odor tuning in afferents (*Or22a*-expressing OSNs) and in the PNs that share the same glomerulus (DM2) in *Drosophila melanogaster* (see Wilson et al. 2004). One observes that, a few hundred ms after onset of antennal lobe activity, the DM2 PNs respond to more odors than the afferents to which they are connected. This result is consistent with the general observation that, in *Drosophila* as well as in locust, PNs respond on average to about 50% of the odors presented to them. Response patterns of individual PNs are generally different for different odors. (c) Diagrams indicating the proposal that broader PN tuning affords better occupancy of coding space across PNs than across afferents. Sharply tuned OSNs can only indicate the presence or absence of the chemicals that activate them. To take an analogy, color vision with three sharply tuned and non-overlapping cone photopigments would not allow the discrimination of hues permitted by primate trichromacy. A system of broadly tuned neurons with overlapping sensitivities allows for better coverage of the input space and higher discriminability (see text).

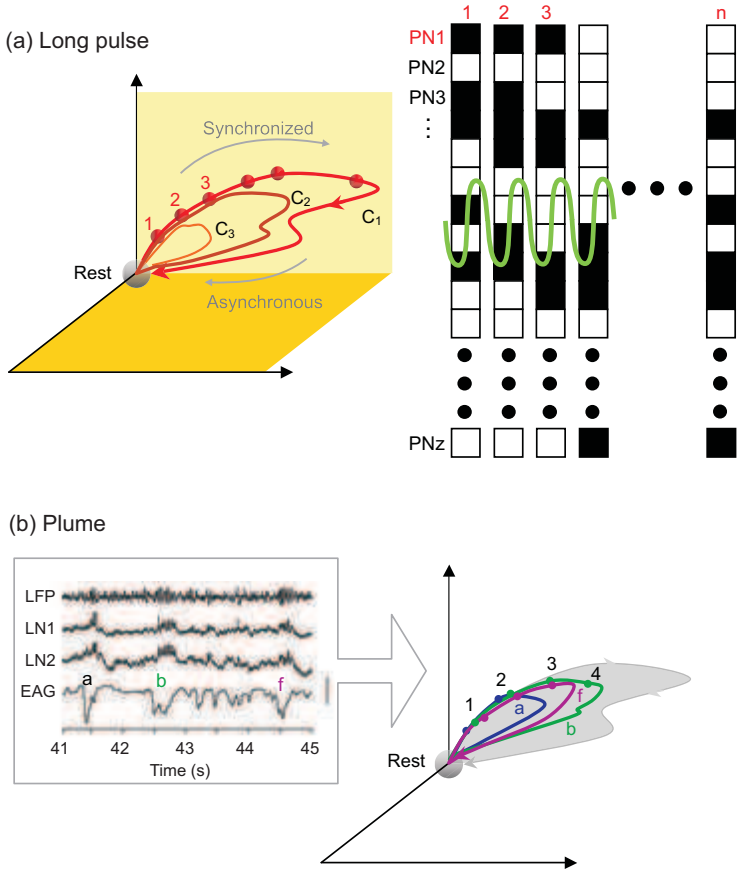

Figure 10.3 Schematic representation of projection neuron (PN) dynamics in the antennal lobe (AL). (a) Abstract representation of odor-evoked PN population activity patterns as trajectories in phase space; on the left, each dimension or axis can be thought of as plotting the instantaneous firing rate of a PN. In locust, this phase space thus has 830 dimensions. Using dimensionality reduction techniques (see text), this high-D space can be projected onto 2 or 3 and are thus depicted. $C_{1–3}$: trajectories evoked by different concentrations of the same odors. Each point on each trajectory (e.g., points labeled 1, 2, 3) corresponds to a vector of activity across the PN population at a given oscillation cycle. These vectors are schematized on the right, where each column represents the state of the PN population (one PN per box; off: white; on: black) and the successive columns represent the evolution of these PN vectors over successive cycles of the oscillatory AL output. Different concentrations of the same odor evoke a family of related trajectories, forming low-dimensional manifolds in the original phase space, specific for each odor. (b) In an odor plume, each odor-evoked trajectory is interrupted early, due to the short duration of odor filaments (EAG: electroantennogram; LFP: local field potential; LN: local neuron intracellular recordings, M. Stopfer and G. Laurent, unpublished). Trajectories a, b, and f (right) schematize the spatiotemporal activity patterns evoked by filaments a, b, f on data at left. Whether the fragments of PN trajectory caused by plume stimuli lie on the manifolds that can be defined with controlled odor pulse stimuli is still not known.

show successions of short bursts of activity. Those can be depicted as sequences of short trajectories (a–f) that would lie on the manifolds defined with long odor pulses. The longer the odor filament, the greater the number of oscillation cycles it forces the projection neuron population to go through. The intermittency of stimulation inherent to a plume results in a sequence of trajectories.

DECODING THE PROJECTION NEURON POPULATION OUTPUT

Connectivity

Antennal lobe projection neurons project to the mushroom body where they send distributed collateral projections throughout the calyx. In locust, each projection neuron was estimated to make over 600 (possibly as many as 2,000) output synapses (Perez-Orive et al. 2002). [More recent results (Jortner, Farivar, and Laurent, in preparation) indicate that connectivity between projection neurons and Kenyon cells is in fact a lot more widespread, but the reasoning presented below still applies.] Our early estimates come from anatomical analysis of projection neuron axonal projections and from electron microscopy of axonal presynaptic varicosities (Leitch and Laurent 1996). If we suppose that each projection neuron makes 1200 individual outputs on average, the projection neuron population makes a total of 1200×830 output synapses in the calyx. If those synapses are made onto all 50,000 Kenyon cells (i.e., if all Kenyon cells receive olfactory information), each Kenyon cell must be connected to 20 projection neurons on average (Figure 10.4). This estimate may vary across the Kenyon cell population. Our recent estimates, based on paired electrophysiological recordings, are that connectivity between projection neurons and Kenyon cells is, in fact, so dense that any projection neuron has about 50% chance of being connected with any one Kenyon cell (Jortner, Farivar, and Laurent, in preparation). The gain of any such synapse is, however, very small on average, and the firing threshold of a Kenyon cell requires the coactivation of 100–200 presynaptic projection neurons.

In addition, because projection neurons have widespread projection throughout the calyx, any Kenyon cell has access to most (if not all) projection neuron axons' terminals. This suggests that individual Kenyon cells could detect inputs across any possible combination of projection neurons. However, the number of possible combinations of 400 projection neurons among 830 is tremendously large ($\sim 10^{240}$). Given that there are only 50,000 Kenyon cells, and if we assume that the distribution of connections is random (we do not know this to be true, but projection neuron axonal projections fail to reveal any gross bias), we conclude that those 50,000 realized combinations must be very different from one another. Herein may reside the key to sparseness and associativity among Kenyon cells. For any one odor, rare will be the Kenyon cells whose inputs

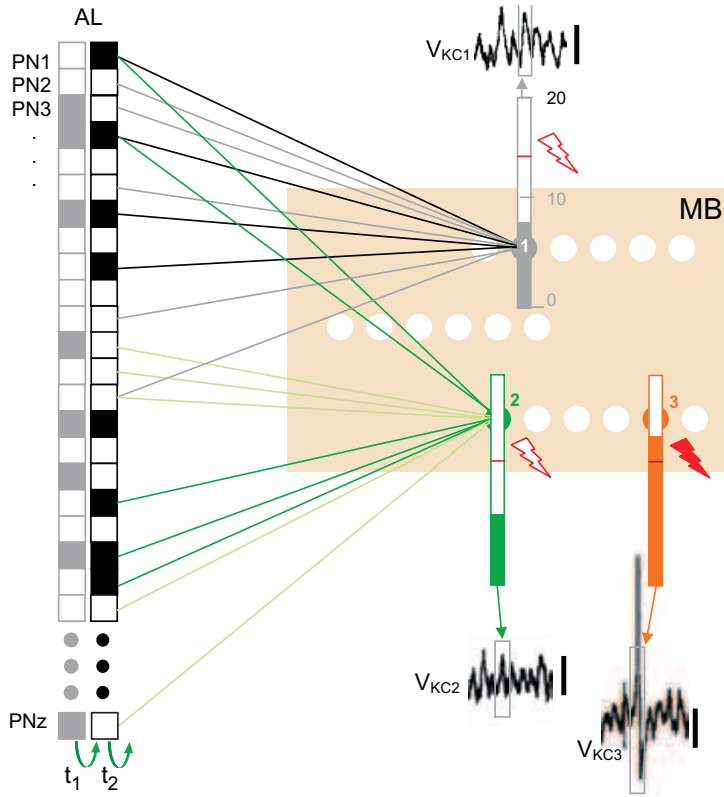

Figure 10.4 Connectivity between antennal lobe (AL) projection neurons (PNs) and mushroom body (MB) Kenyon cells (KCs). Recent results (Jortner, Farivar and Laurent, in preparation) suggest that each KC receives direct excitatory inputs from about 50% of all PNs on average. Similarly, individual PNs diverge to thousands of KCs. Each KC is thus characterized by the complement of PNs converging onto it and acts as a detector of the coactivation of a fraction of these PNs during each oscillation cycle. The threshold (in terms of number of coactive PNs) for each KC may vary across KCs and across time for each KC. In this illustration, KC3 reaches firing threshold; the others do not. The membrane potentials of all KCs, however, indicate periodic PN input, caused by the subsets of PNs presynaptic to each of them. (Intracellular data from Laurent and Naraghi 1994.) Simple combinatorics show that this distributed pattern of connections between PNs and KCs may in great part explain the KCs' high selectivity and, thus, the sparseness of odor representations in the MBs (see text for details).

match precisely enough the projection neuron activity vectors occurring at each oscillation cycle. At present, the actual connection matrix is beyond experimental determination, although it is possibly within reach in *Drosophila*, which has $\sim$200 projection neurons and 2500 Kenyon cells on each side of the brain. Figure 10.4 indicates some of the basic features that can explain Kenyon cell odor specificity in locust:

- Each Kenyon cell is connected to specific set of projection neurons (see above).
- Each Kenyon cell is silent at rest (Perez-Orive et al. 2002).
- Each Kenyon cell has a high firing threshold (Perez-Orive et al. 2002).
- Each Kenyon cell has a limited integration time constant (about half an oscillation cycle; Laurent and Naraghi 1994; Perez-Orive et al. 2002). This property is explained in Figure 10.5.

During an odor, each oscillation cycle is caused by a combination of active projection neurons. Each Kenyon cell, by virtue of its particular input set, detects projection neuron coincidence if enough of the projection neurons presynaptic to it are coactive during that cycle. For most Kenyon cells and cycles, too few presynaptic projection neurons are active, leading only to subthreshold responses. Kenyon cells spikes are extremely rare, but subthreshold membrane potential oscillations are always seen. Kenyon cells thus act as binary classifiers of projection neuron activity vectors: When a projection neuron activity pattern matches closely enough the projection neuron connection pattern of one Kenyon cell, that Kenyon cell can reach threshold and signal the occurrence of that projection neuron combination. This selective detection is repeated at each oscillation cycle.

Integration Window

Critical to the Kenyon cell's specificity, and thus to the sparseness of odor representations in the mushroom bodies, is their short temporal integration window (about one half of each oscillation cycle). We know at least two different mechanisms that ensure the reset of each Kenyon cell before the start of a new oscillation cycle, thus curtailing temporal summation: feedforward inhibition and voltage-gated dendritic properties.

Delayed, Nonspecific, Feedforward Inhibition (Figure 10.5a)

In addition to sending distributed axonal collaterals to the mushroom body, all antennal lobe projection neuron axons terminate in the lateral horn; there, they contact a population of about 60 GABAergic interneurons, whose own axons terminate in the mushroom body calyx, providing feedforward inhibition to Kenyon cells. Because 830 projection neurons converge on a small population of lateral horn interneurons (LHI), LHI responses to odors are oscillatory, precisely locked to projection neuron output, but 180° out of phase with it (Perez-Orive et al. 2002). Because each LHI receives inputs from many projection neurons, LHI responses are poorly odor specific. This has important consequences for individual Kenyon cells: By virtue of its connections to only a fraction of all projection neurons, the depolarization of a Kenyon cell is critically dependent on the coactivation of many of its presynaptic projection neurons.

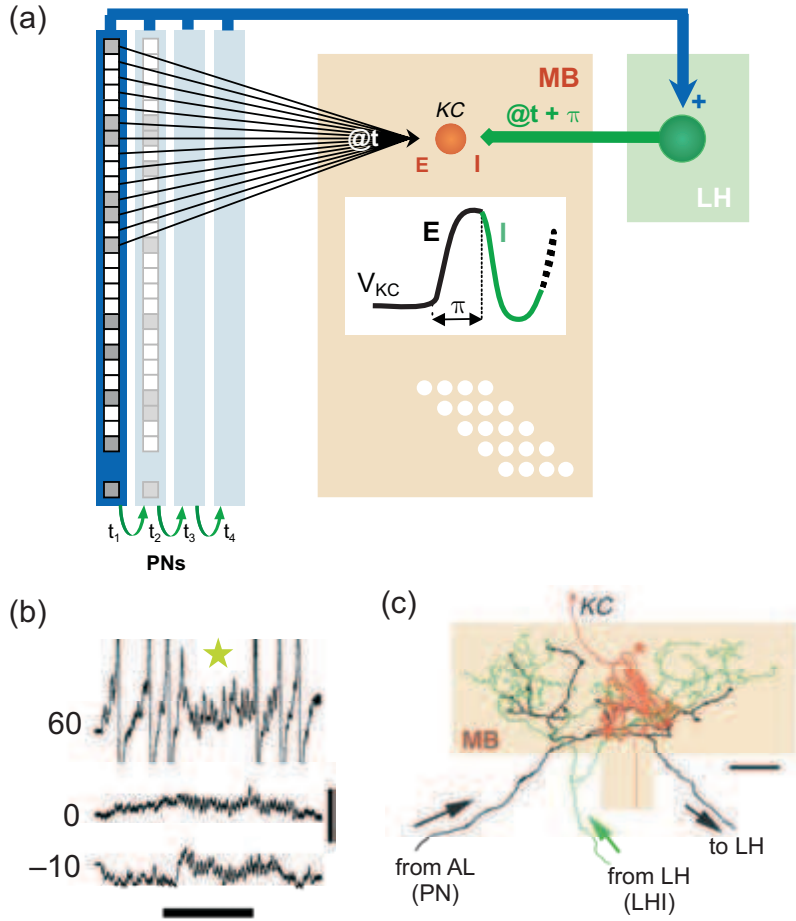

Figure 10.5 Decoding of periodic and synchronized PN input by mushroom body (MB) Kenyon cells (KCs). (a) Cellular and circuit mechanisms responsible for low KC response probability and high input specificity. At each oscillation cycle, each KC receives a volley of excitatory PN inputs (E) from the large subset of PNs to which it is connected. The correspondence between the set of anatomical PN inputs to a KC and the PN activity vector at that time determines the level of depolarization of that KC. The PNs also indirectly mediate feedforward inhibition (I) of the KCs via a small population of lateral horn interneurons (LHIs). LHI activity is π out of phase with PN activity; hence, each wave of PN excitation received by a KC at one oscillation cycle is curtailed by a wave of inhibition, one half of an oscillation cycle later. Kenyon cell temporal integration is thus limited to short periods. See Perez-Orive et al. (2002) for more details. (b) Intracellular recording from a KC during an odor response (bar), while the KC is held at rest (0) or polarized with direct current (–10pA, +60pA). Note the inhibitory interruption of DC-induced spiking (by LHI feedforward inhibition) and the amplification of PN-evoked EPSPs (by voltage-gated properties) in the KC when the neuron is held depolarized. (c) Composite drawing showing KC dendrites, PN axon and LHI axon in a schematized MB calyx. See Perez-Orive et al. (2002) for details.

For each oscillation cycle, the compound EPSP ("E" in Figure 10.5a) to each Kenyon cell is hence tightly odor dependent. By contrast, because LHI responses are not odor specific, the compound IPSP ("I" in Figure 10.5a) that follows each EPSP in a Kenyon cell is reliable. It acts as a periodic reset, preventing temporal integration over successive oscillation cycles. Correspondingly, blocking the IPSP pharmacologically enables temporal summation well beyond single oscillation cycles by Kenyon cells and abolishes their specificity (Perez-Orive et al. 2002).

Voltage-dependent Properties of Kenyon Cell Dendrites (Figure 10.5b)

Intracellular recordings from Kenyon cells during odor responses reveal that the EPSPs they receive are periodically amplified if the Kenyon cell is held depolarized by direct current injection (e.g., 60 pA, Figure 10.5b). This amplification is voltage dependent and considerably shortens the decay time constant of the EPSP (Laurent and Naraghi 1994; Perez-Orive et al. 2002, 2004). This shortens the summation window for projection neuron input and increases the Kenyon cells' selectivity for coincident projection neuron input.

The Basic Projection Neuron–Kenyon cell–LHI Circuit (Figure 10.5c)

This figure is a camera lucida drawing constructed from intracellular stains of projection neuron (black) and LHI (green) axons and one Kenyon cell (red) in a schematized mushroom body calyx. All stains were carried out in separate brains and the drawing is a composite of three separate fills. This figure illustrates the two principal components of the local excitatory and inhibitory circuits that shape the highly selective responses of Kenyon cells.

Building Invariance

Whereas the response patterns of individual projection neurons change (sometimes dramatically) with concentration, projection neuron activity vectors (*spatiotemporal patterns* across the projection neuron population) change progressively with concentration, preserving clear similarities between the population responses corresponding to different odor concentrations. This can be seen in the projections of these families of trajectories in a low-dimensional space, using techniques such as "locally linear embedding or principal components analysis" (Stopfer et al. 2003; schematized in Figures 10.2 and 10.6a). Intracellular or tetrode recordings of Kenyon cells (Figure 10.6b), by contrast, indicate that Kenyon cell sensitivity to concentration is different: of those Kenyon cells that respond to a given odor, some are sensitive to a very narrow concentration range (e.g., KC5, Figure 10.6c), whereas others respond equally well over several orders of magnitude of concentration, thus expressing ranges of concentration invariance (e.g., KCs 1 and 3, Figure 10.6c). A given odor stimulus at a

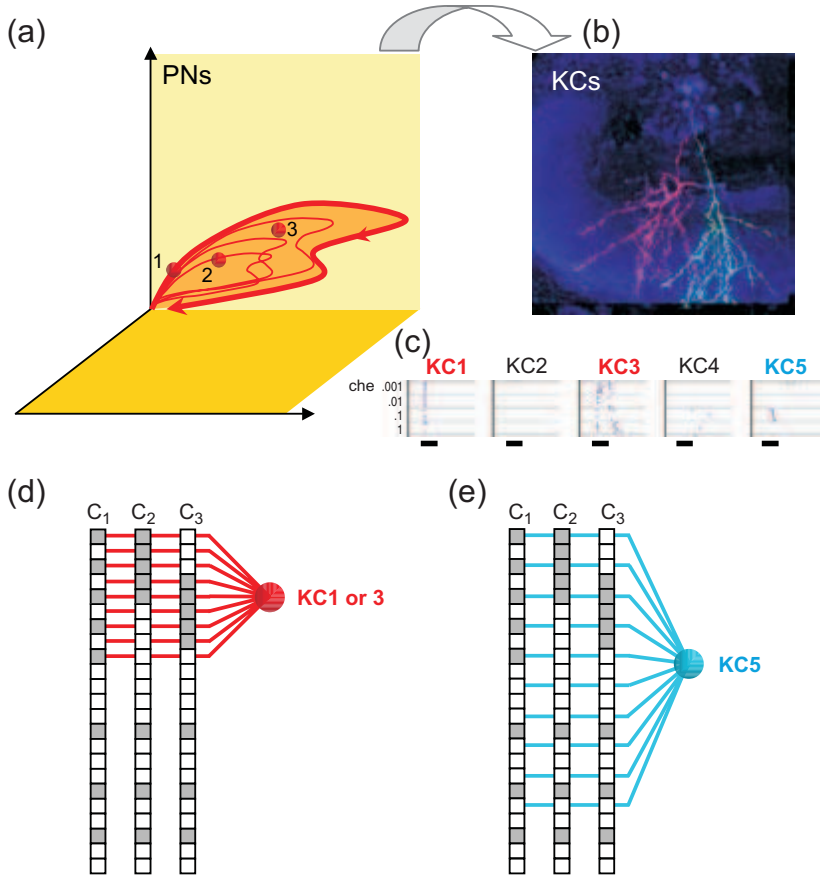

Figure 10.6 Hypothesis for the mechanisms underlying the construction of invariant properties. (a) Family of trajectories representing projection neuron (PN) response patterns for different odor concentrations (from Stopfer et al. 2003). This display illustrates the high correlations between the PN activity patterns evoked by different concentrations of an odor. See Figure 10.3 and text for details on construction. (b) Simultaneous intracellular fills of two Kenyon cells (KCs) in the locust mushroom body calyx (courtesy of S. Farivar). (c) Simultaneous recordings from five KCs, showing two invariant cells (KC1, 3) and one concentration-specific cell (KC5) (see Stopfer et al. 2003). There are thus concentration-invariant cells within the KC population. (d) Hypothetical connectivity between PNs and one KC such that the KC would respond to all three concentrations ($C_{1–3}$). See text for details. (e) Hypothetical connectivity between PNs and this KC, such that it would respond only to C_1. In both cases and for the sake of illustration, the KC firing threshold is arbitrarily set at 5 simultaneous PN inputs. In reality, this threshold is estimated to be between 100 and 200 (see text for details).

particular concentration thus activates a Kenyon cell assembly that will contain both "identity encoders" (the concentration-invariant Kenyon cells) and more specific elements, sensitive also to concentration (Stopfer et al. 2003). This

invariance is very interesting because it corresponds to "high-level" properties generally attributed to brain areas many layers away from sensory inputs. Here we are only two synapses away from the olfactory receptor neurons and thus have an opportunity to study without too much difficulty the mechanisms underlying the "construction" of such properties.

How Could One Generate a Concentration-invariant Cell?

A possible solution derives directly from the mechanisms thought to underlie the decoding of projection neuron output by Kenyon cells: because (a) different concentrations of an odor evoke different, but related projection neuron spatiotemporal response patterns, (b) individual Kenyon cells sample a large subset of all the projection neurons (we presently estimate these subsets to be 50% of the projection neuron population on average, see section on ODOR REPRESENTATIONS IN THE ANTENNAL LOBES AND MUSHROOM BODIES), and (c) Kenyon cell integration is limited to single oscillation cycles, it follows that the specificity of a Kenyon cell's response depends (a) on the identity of the projection neurons to which it is connected, (b) on their own probability of coactivation (itself determined by the stimulus), and (c) on the firing threshold of the Kenyon cell. If, for example, a Kenyon cell receives a large number of projection neuron inputs and if its own firing threshold is high enough to ensure selectivity, but low enough to allow many different subsets of projection neurons to excite it, one can imagine connection schemes in which many projection neurons activated by one odor over many concentrations converge on the same Kenyon cell. This is illustrated in Figure 10.6d, where a hypothetical Kenyon cell is connected to ten projection neurons that are activated differently, but in an overlapping manner, by three different odor concentrations ($C_{1–3}$) (in a given oscillation cycle) of an odor: by virtue of its connections to these projection neurons, the Kenyon cell is equally activated by all three stimuli and is concentration invariant. By contrast, the hypothetical Kenyon cell in Figure 10.6e is, by virtue of its own connectivity to projection neurons, best activated by concentration 1 of this odor, and thus not invariant. One can see how such a scheme would fail if Kenyon cells were able to integrate over time. A key lies in the projection neuron–Kenyon cell connection matrix (about which we still know little) and on cycle-by-cycle integration: both features ensure high selectivity and exploit the cycle-by-cycle similarity of projection neuron output over concentrations of single odors. To test this hypothesis, we need to characterize precisely the properties of all (or many of) the projection neurons that converge onto one particular concentration-invariant Kenyon cell. This is at present beyond our experimental abilities.

Turbulent Odor Plumes

Odors in the natural world often come in complex, chaotic plumes as a result of the turbulent behavior of the fluids (air or water) by which they are carried. The

odor-coding principles that I have proposed and summarized above are derived from experiments in which odors were pulsed for many hundreds of milliseconds, in a continuous and reliable fashion. How do these nervous systems and olfactory circuits behave when odor delivery is turbulent, uncontrolled, and intermittent? This question was partly answered by experiments in which experimental animals were placed in a turbulent wind tunnel and challenged with odors delivered from a distance of ca. 1 m (Stopfer and Laurent, unpublished).

The example in Figure 10.7a combines simultaneous recordings of an electroantennogram (which monitors bulk receptor activity), LFP from the ipsilateral mushroom body (which monitors projection neuron population input to the mushroom body), and tetrode recording from one Kenyon cell in an intact locust placed in the wind tunnel in the presence of a complex odor (a cherry odor mixture). The electroantennogram's negative deflections indicate odor hits as detected by the receptor population. Note that, for many such deflections, one observes a corresponding short burst of LFP oscillation and, for a smaller fraction of the electroantennogram signals, single spikes from a Kenyon cell specific to this odor (cherry). The same Kenyon cell was then tested with the same odor, but with controlled odor pulses delivered in the classical manner. Figure 10.7b shows its responses to 100 ms long odor puffs at five concentrations. This Kenyon cell showed concentration invariance over a 100-fold concentration range. Its responses to controlled odor pulses were thus consistent with those observed under turbulent odor delivery. To understand the relationship between the results of controlled and turbulent odor delivery conditions better, we then assessed whether each Kenyon cell spike recorded in plume conditions could be correlated, as we would predict from controlled experiments, with the occurrence of an electroantennogram deflection (indicating encounter with an odor filament) and of an LFP oscillation burst (indicting synchronized projection neuron activity). Figures 10.7c and 10.7d plot, respectively, electroantennogram and LFP traces as triggered by every Kenyon cell spike recorded during a 3 minute stretch of turbulent odor plume presentation (as in Figure 10.7a). Each spike from this Kenyon cell was indeed highly correlated with an odor filament hit and with a burst of LFP oscillation. The integrative mechanisms we derived from controlled odor pulse experiments may thus explain how, after processing through the antennal lobe, information distributed across projection neuron populations is synthesized into highly specific high-order responses, informing the animal of repeated hits with a particular odor.

SYNTHESIS

Do the pieces assemble in a way that makes some sense? One remarkable feature of Kenyon cells is their high stimulus specificity. The Kenyon cell in Figure 10.7, for example, responds to a complex fruity blend, which activates hundreds of projection neurons in the antennal lobe. More generally, this system appears

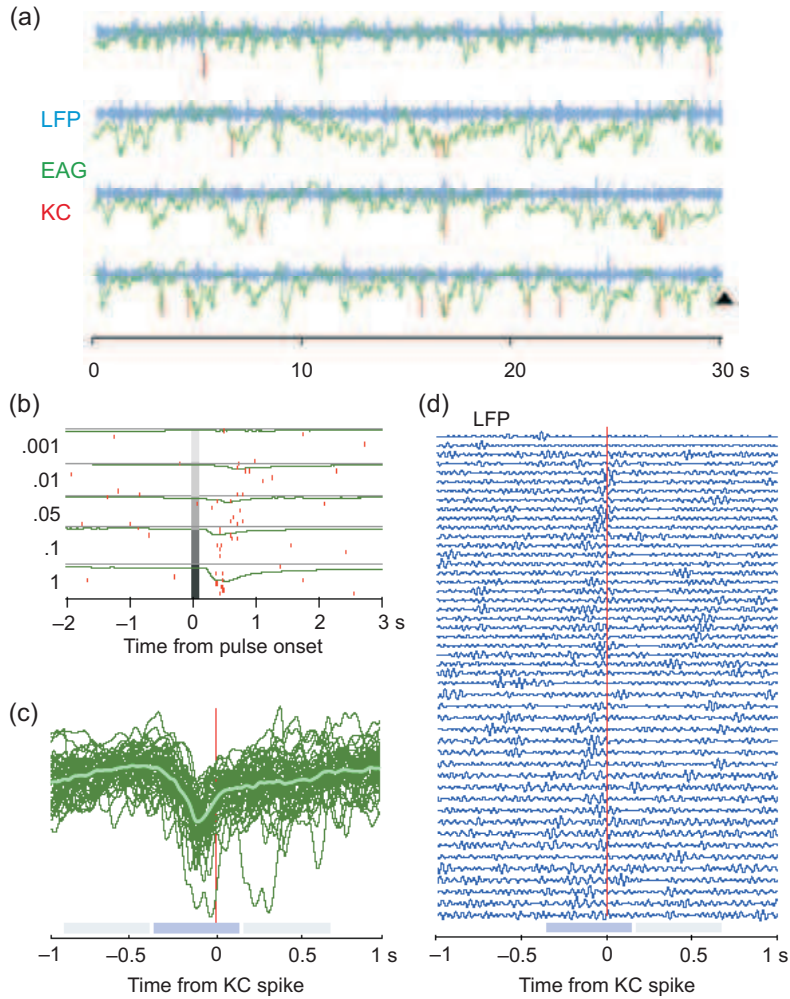

Figure 10.7 Decoding of projection neuron (PN) input in real time by single Kenyon cells (KCs) in odor plumes. (a) Simultaneous local field potential (LFP), electroantennogram (EAG), and KC tetrode recordings in a turbulent odor plume (Stopfer and Laurent, unpublished). Note the rare KC spikes, coinciding with some of the EAG negative deflections, each indicative of an encounter with an odor filament (cherry odor). (b) Same KC and EAG as in (a), but now in response to controlled odor pulses of increasing concentrations (0.001 to 1; ten trials per concentration). Note broad concentration tuning of this KC. (c) Superimposed EAG traces, triggered on the KC spikes produced during plume stimulation as in (a). Note the tight correlation between the two signals, indicating that the KC fires on average about 100 ms after the peak of an EAG deflection and that its spikes reliably predict the occurrence of a hit with this odor. (d) LFP traces triggered on the KC spikes, recorded in a turbulent plume. Note the LFP oscillatory burst around each KC spike, indicating that each spike coincides with a volley of synchronized PN input caused by each odor filament.

to enable highly specific detection/recognition of complex odor patterns, only two synapses downstream from the receptors. At the population scale, it is equivalent to a sparsening of stimulus representations. This represents the solution to a pattern recognition problem and, thus, potentially has broad significance. Some relevant propositions are:

- *The antennal lobe acts as a mixing/formatting box*, which means that projection neuron "tuning" can be broader than that of the olfactory afferents to which they are directly connected. There appears to be very little sharpening of single-cell properties. This makes sense if one accepts that at this stage, odor representations are carried by large neuron populations. For many distributed systems of representation, a sparseness of 0.5 (broad tuning) is optimal.
- *To understand the code, study the decoder.* Most of the apparent complexity of neuronal activity in the antennal lobe begins to make sense once one considers the antennal lobe's principal target: the mushroom body. Its principal neurons, the Kenyon cells, are one of the two decoders of this output. Their properties reveal the relevant temporal and spatial scales for decoding projection neuron output.
- *Temporal formatting of projection neuron output* occurs on at least two timescales: a slow timescale, which may represent the consequences of distributing and optimizing activity across projection neurons, and a fast timescale, which represents the creation of discrete packets of projection neuron output, once per oscillation cycle.
- *Decoding of projection neuron output by Kenyon cells*, from a multitude of across-projection neuron combinations, is made possible by numbers and connectivity. In all known insect olfactory systems, there are always many more Kenyon cells (thousands to hundreds of thousands) than there are projection neurons (hundreds); output from individual projection neurons is distributed to many Kenyon cells over the entire mushroom body; Kenyon cells seem to receive input from many projection neurons each. Each Kenyon cell is assigned one particular projection neuron input pattern. However, if, as we measured in locusts (Jortner, Farivar, and Laurent, in preparation), each Kenyon cell samples a very large fragment of the projection neuron population (possibly one half), there are not enough Kenyon cells to realize all possible combinations of projection neurons (there are $\sim$10,240 possible combinations of 400 projection neurons among 800). If we assume random (or near-random or pseudo-random) assignment of connections between projection neurons and Kenyon cells, we conclude that each Kenyon cell must receive a projection neuron combination that differs greatly, on average, from those of all the other Kenyon cells. In other words, each Kenyon cell's input set is unique and (if

connection probability between individual projection neurons and Kenyon cells is 0.5) maximally different on average from those of the other Kenyon cells. This may explain why Kenyon cell responses are so specific and, thus, how sparseness arises. At the same time, the system requires a large enough population of Kenyon cells to encode, in this sparse manner, a large fraction of all possible projection neuron combinations. This excess of Kenyon cells (relative to projection neurons) enables their population to sample a large number of possible odors. If the firing threshold of each Kenyon cell is adjusted properly so that it can be activated by a family of related projection neuron patterns (i.e., if the Kenyon cell's firing threshold is much lower than the total number of projection neuron inputs it receives), this Kenyon cell can become concentration invariant; this is because the sets of projection neurons activated by different concentrations of an odor are correlated or overlapping (Stopfer et al. 2003). Connectivity and cycle-by-cycle decoding rules could thus provide invariance.

- *Single-cycle decoding*: locust Kenyon cells are tuned to prevent temporal summation using both feedforward inhibition and intrinsic biophysical properties. Kenyon cells have a "membrane memory," which does not extend much beyond one oscillation cycle. (It should be noted, however, that if projection neuron-to-Kenyon cell synapses display short-term plasticity, the effects of the projection neuron input patterns occurring over successive oscillation cycles might not be truly independent from one another; this could be an interesting way of biasing Kenyon cell selectivity.) This also sharpens their specificity: provided their firing threshold is sufficiently high, Kenyon cells fire only upon simultaneous coactivation of a high proportion of their presynaptic projection neurons. Thus far, our results suggest that each Kenyon cell receives input from 1/2 of the projection neuron population (on average), and that its firing threshold corresponds to the coactivity of between 1/4 and 1/2 of each set of converging projection neurons. These observations raise many interesting questions related to the optimality of convergence and divergence ratios, firing thresholds, and adaptation. Clearly, such values must be precisely adapted and the role of learning to achieve this is so far not known.
- *Similarity with linear classifiers*: many of the features shown by antennal lobe–mushroom body interactions are surprisingly reminiscent of linear classifiers developed for pattern recognition in engineering. Support vector machines (Vapnik 1998), for example, map their n-dimensional input space nonlinearly into a high-dimensional feature space in which a linear classifier is then constructed. In this analogy, the Kenyon cells act as the linear classifiers, cutting up feature space into separated features; Kenyon cell connectivity defines the hyperplanes doing the separation.

POSSIBLE TOPICS FOR DISCUSSION

I have deliberately focused the discussion in this chapter on our recent work—the interpretations of which are working hypotheses, obviously open fully to criticism, rejection, or refinement—in the spirit of the Dahlem Workshops. To facilitate further discussion, I presented results and interpretations in separate, though linked sections. Potentially interesting discussion topics could relate to data: Can we all agree on some body of experimental facts? If so, which are they? Where are there differences? What do they tell us? Other questions concern interpretation:

1. The description of response dynamics (slow temporal patterns and LFP oscillations) is not new. Both were shown to exist many decades ago by Adrian (1942), for oscillations, and by Macrides and Chorover (1972), Meredith (1986), Kauer and Moulton (1974), and others for slow response patterning, both in the mammalian olfactory bulb. Our work analyzes those known phenomena and attempts to explain their causes and consequences for brain function. Recent results in related systems, however, dispute the existence of oscillatory synchronization (Lei et al. 2002) or its coherence across olfactory networks (Christensen et al. 2003). The causes for these discrepancies should be analyzed.
2. How does recent imaging work in honeybees (Galizia et al. 1999) and flies (Ng et al. 2002; Fiala et al. 2002; Wang et al. 2003) match or differ from the physiological picture we propose? In particular, recent molecular imaging in flies (Wang et al. 2003) suggests that the antennal lobe faithfully transmits olfactory receptor information. This poses a discrepancy with our physiological results on flies and locusts (Wilson et al. 2004) and might be explained by technical differences in methods for observation.
3. An old interpretation of local inhibition in the olfactory bulb is that it underlies the sharpening of single mitral cell tuning by lateral inhibition between similarly tuned input channels (Mori and Shepherd 1994). The existing evidence in support of or against this hypothesis as well as its rationale could be discussed. Similarly, if our contention that antennal lobe circuits distribute afferent activity is correct, what is the neuronal substrate for such lateral processing?
4. More broadly, the possible roles played by inhibition (e.g., sharpening of single-cell tuning, normalization and gain control, oscillatory synchronization, pattern generation) could be discussed.
5. It has been suggested (Yoshihara 2001) that the temporal complexity of locust or zebrafish antennal lobe/olfactory bulb output is due to their containing multiglomerular (as opposed to uniglomerular) principal neurons. This does not seem to be borne out by data, but could be examined.

6. Recent results in zebrafish olfactory bulb extend the insect results in suggesting multiplexing functions for oscillatory synchronization (Friedrich and Laurent 2001; Friedrich et al. 2004). This work again emphasizes the need to understand decoding mechanisms by circuits downstream of the olfactory bulb.

7. Our physiological and behavioral results on honeybees and locusts (Stopfer et al. 1997; MacLeod et al. 1998) suggested that oscillatory synchronization might be useful for fine, but not for coarse, discrimination. In view of our more recent results on decoding of oscillatory output, I would propose that the bee experiments may not have addressed the fundamental issue. The key issue could instead be one of memory capacity. The mushroom bodies are clearly involved in olfactory memory, and the format of odor representations is, on theoretical grounds, ideal for associative memories. I venture that the degradation of antennal lobe output (as caused by desynchronization) and the decrease in mushroom body representation sparseness it causes (Perez-Orive et al. 2002) ultimately increases the probability of representation overlap and thereby decreases capacity. How this could be tested remains to be discussed.

8. Recent behavioral results in humans (Wise and Cain 2000), rats (Uchida and Mainen 2003), and honeybees (Ditzen et al. 2003) indicate that odor discrimination can occur in a time window between 300 ms (rats) and 2000 ms (humans), independently of the task's difficulty. What constraints does this impose on physiological results? Are these data compatible with physiology?

9. Some investigators hold the view that a fly's brain tells us little about a mouse's own, maybe with reason. Why do such doubts exist? What are the right levels of description and understanding (implementation details vs. algorithms and rules) that will increase the chances of cross-fertilization?

10. Is work focused on olfactory circuits likely applicable to other sensory modalities or other functional networks? What systems might olfactory work naturally relate to?

REFERENCES

Adrian, E. 1942. Olfactory reactions in the brain of a hedgehog. *J. Physiol. (Lond.)* **100**:459–473.

Buck, L.B. 1996. Information coding in the vertebrate olfactory system. *Ann. Rev. Neurosci.* **19**:517–544.

Buck, L.B., and R. Axel. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* **65**:175–187

Christensen, T.A., H. Lei, and J.G. Hildebrand. 2003. Coordination of central odor representations through transient, non-oscillatory synchronization of glomerular output neurons. *PNAS* **100**:11,076–11,081.

Ditzen, M., J.F. Evers, and C.G. Galizia. 2003. Odor similarity does not influence the time needed for odor processing. *Chem. Senses* **28**:781–789.

Fiala, A., T. Spall, S. Diegelmann, et al. 2002. Genetically expressed cameleon in *Drosophila melanogaster* is used to visualize olfactory information in projection neurons. *Curr. Biol.* **12**:1877–1884.

Freeman, W.J. 1978. Spatial properties of an EEG event in the olfactory bulb and cortex. *Electroencephalogr. Clin. Neurophysiol.* **44**:586–605.

Friedrich, R.W., C.J. Habermann, and G. Laurent. 2004. Multiplexing using synchrony in the zebrafish olfactory bulb. *Nat. Neurosci.* **7**:862–871.

Friedrich, R.W., and G. Laurent. 2001. Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity. *Science* **291**:889–894.

Galizia, C.G., S. Sachse, A. Rappert, and R. Menzel. 1999. The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. *Nat. Neurosci.* **2**:473–478.

Hildebrand, J.G., and G.M. Shepherd. 1997. Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla. *Ann. Rev. Neurosci.* **20**:595–631.

Kauer, J.S. 1987. Coding in the olfactory system. In: Neurobiology of Taste and Smell, ed. T.E. Finger and W.L. Silver, pp. 205–222. New York: Wiley.

Kauer, J.S., and D. Moulton. 1974. Responses of olfactory bulb neurones to odour stimulation of small nasal areas in the salamander. *J. Physiol. (Lond.)* **243**:717–737.

Laurent, G., and M. Naraghi. 1994. Odorant-induced oscillations in the mushroom bodies of the locust. *J. Neurosci.* **14**:2993–3004.

Lei, H., T.A. Christensen, and J.G. Hildebrand. 2002. Local inhibition modulates odor-evoked synchronization of glomerulus-specific output neurons. *Nat. Neurosci*. **5**:557–565.

Leitch, B., and G. Laurent. 1996. GABAergic synapses in the antennal lobe and mushroom body of the locust olfactory system. *J. Comp. Neurol*. **372**:487–514.

MacLeod, K., A. Backer, and G. Laurent. 1998. Who reads temporal information contained across synchronized and oscillatory spike trains? *Nature* **395**:693–698.

Macrides, F., and S.L. Chorover. 1972. Olfactory bulb units: Activity correlated with inhalation cycles and odor quality. *Science* **185**:84–87.

Menzel, R. 1987. Memory traces in honeybees. In: Neurobiology and Behaviour of Honeybees, ed. R. Menzel and A. Mercer, pp. 310–325. Berlin: Springer-Verlag.

Meredith, M. 1986. Patterned response to odor in mammalian olfactory bulb: The influence of intensity. *J. Neurophysiol.* **56**:572–597.

Mombaerts, P., F. Wang, C. Dulac et al. 1996. The molecular biology of olfactory perception. *Cold Spring Harb. Symp. Quant. Biol.* **61**:135–145

Mori, K., and G.M. Shepherd. 1994. Emerging principles of molecular signal processing by mitral/tugted cells in olfactory bulb. *Sem. Cell. Biol*. **5**:65–74.

Ng, M., R.D. Roorda, S.Q. Lima et al. 2002. Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. *Neuron* **36**: 463–474.

Perez-Orive, J., O. Mazor, G.C. Turner et al. 2002. Oscillations and sparsening of odor representations in the mushroom body. *Science* **297**:359–365.

Perez-Orive, J., M. Bazhenov, and G. Laurent. 2004. Intrinsic and circuit properties favor coincidence detection for decoding oscillatory input. *J. Neurosci.* **24**:6037–6047.

Singer, W., and C. Gray. 1995. Visual feature integration and the temporal correlation hypothesis. *Ann. Rev. Neurosci*. **18**:555–586.

Stopfer, M., S. Bhagavan, B.H. Smith, and G. Laurent. 1997. Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. *Nature* **390**:70–74.

Stopfer, M., V. Jayaraman, and G. Laurent. 2003. Intensity versus identity coding in an olfactory system. *Neuron* **39**:991–1004.

Uchida, N., and Z.F. Mainen. 2003. Speed and accuracy of olfactory discrimination in the rat. *Nat. Neurosci*. **6**:1224–1229.

Vapnik, V. 1998. Statistical Learning Theory. New York: Wiley.

Vosshall, L.B., H. Amrein, P.S. Morozov, A. Rzhetsky, and R. Axel. 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**:725–736.

Vosshall, L.B., A.M. Wong, and R. Axel. 2000. An olfactory sensory map in the fly brain. *Cell* **102**:147–159.

Wang, J.W., A.M. Wong, W. Flores, L.B. Vosshall, and R. Axel. 2003. Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. *Cell* **112**: 271–282.

Wilson, M.A., and B.L. McNaughton. 1993. Dynamics of the hippocampal ensemble code for space. *Science* **261**:1055–1058.

Wilson, R.I., G.C. Turner, and G. Laurent. 2004. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science* **303**:366–370.

Wise, P.M., and W.S. Cain. 2000. Latency and accuracy of discriminations of odor quality between binary mixtures and their components. *Chem. Senses* **25**:247–265.

Yoshihara, Y., H. Nagao, and K. Mori. 2001. Sniffing out odors with multiple dendrites. *Science* **291**:835–837.

11

Neuronal Replacement in the Adult Olfactory Bulb Microcircuits

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ABSTRACT

The central nervous system is made up of many specific types of neurons intricately intertwined to form complex neuronal networks. In the cortex, networks of inhibitory interneurons play a crucial role in modulating the electrical activity patterns of the principal neurons known as projecting (glutamatergic) neurons. The large diversity of local-circuit inhibitory interneurons containing γ-aminobutyric acid (GABA) reflects their different functions within cortical networks. Identifying and defining the characteristic features of the inhibitory neurons is, therefore, essential to achieve a cellular understanding of complex brain activities, from perception to cognition.

The olfactory bulb is ideally suited for such physiological and pharmacological investigations: it is readily accessible at the rostral end of the CNS; it is a laminated structure where projecting neurons and interneurons can be easily distinguished; input and output pathways are distinctly located; and the local GABAergic interneurons are replaced continuously in the adult olfactory bulb. How newborn neurons integrate a preexisting neural network and how basic network functions are maintained when a large percentage of neurons are continuously renewed are two important issues that remain to be answered.

A description is provided on how the production of new GABAergic interneurons is adapted to experience-induced plasticity. Such an adaptation is sensitive to the level of sensory inputs and, in turn, neurogenesis may adjust the neural network functioning to optimize sensory information processing. By maintaining a constitutive turnover of bulbar interneurons that is subjected to modulation by environmental cues, the ongoing neurogenesis in the adult is associated with improved sensory and cognitive abilities. This review brings together recently described properties of interneurons as well as emerging principles of their functions that indicate a much more complex role for these cells than just gatekeepers providing inhibition.

INTRODUCTION

From modern neurobiology, we have learned that complex biological systems, such as the vertebrate brain, are built from similar, repeated units. In the CNS, these units, or modules, are composed of networks that have opposite effects: excitation and inhibition. Initially, excitation was assumed to play the dominant role in information processing, whereas inhibition served mainly to eliminate excessive activity. Local inhibitory neurons appeared to be simple elements that regulated excitatory neuronal activity. Thus, as in Yin and Yang philosophy, the activity of a large network of principal excitatory (glutamatergic) neurons was balanced purposefully by local-circuit inhibitory neurons.

Today, it is clear that the inhibitory interneurons occupy distinct positions within brain microcircuits where they form mainly local connections. One of the most popular cell types studied so far in the brain is the γ-aminobutyric acid (GABA)-releasing neuron, also referred to as the local-circuit inhibitory interneuron. Recent investigations indicate that the initial concept of an interneuron is an oversimplification and requires readjustment to accommodate cellular types and functions that do not strictly fit the definition cited above. For instance, combined anatomical, electrophysiological, and pharmacological approaches have recently allowed better insight into the roles played by interneurons at several distinct levels. At the cellular level, such functions range from governing action potential generation, firing pattern, precise timing of the discharge of individual principal cells in relation to the emergent behavior of the entire cell assembly, membrane potential oscillations, and dendritic calcium spikes. At the network level, inhibitory interneurons are considered to be important for controlling synaptic strength and plasticity as well as generating large-scale synchronous oscillatory activity (reviewed in Maccaferri and Lacaille 2003).

Despite the general interest in interneurons, one important caveat that has constantly limited studies of their function has been the tremendous heterogeneity in their morphology and connectivity, as opposed to principal neurons, which are much more uniform in their appearance. Anatomically, interneurons represent one of the most diverse populations in the mammalian CNS. Thus, classifications based solely on cell anatomy tell us little about interneuron function (Maccaferri and Lacaille 2003). Because of this limitation, other attempts to characterize interneuron subtypes have been based on neurochemical content (e.g., calcium-binding proteins or neuromodulators). However, neurochemically identical cells can possess surprisingly different functional properties, and this further complicates classification. Furthermore, since inhibitory interneurons exert pleiotropic actions on brain microcircuits, characterizations based solely on function have proved to be problematic. To be valid, therefore, any classification of interneurons has to take collectively into account the morphological, biochemical, and physiological characteristics. Remarkably, these

criteria are met at the first central relay of sensory odor information, the olfactory bulb. We shall see how this structure is appropriate to explore interneuron function and is subject to plasticity in the adult CNS. Due to space constraints, this review focuses only on the olfactory system of mammals.

THE MAMMALIAN OLFACTORY BULB NETWORK

In mammals, the initial event of odor detection takes place at the peripheral olfactory system: the olfactory epithelium of the nasal cavity. Olfactory transduction then starts with about a thousand different types of odorant receptors, which are located on the cilia of sensory neurons that constitute the olfactory neuroepithelium. The sensory neurons project to a small number of olfactory glomeruli paired on both the medial and lateral aspects of the olfactory bulb (reviewed in Bozza and Mombaerts 2001). About 20 to 50 second-order neurons emanate from each glomerulus and project to a number of higher centers, including the olfactory cortex. Using a transsynaptic tracer expressed in olfactory receptor neurons under the control of two specific olfactory receptor promoters, it has been shown that the projection of bulbar output neurons, which receive sensory inputs from homologous olfactory neurons, form reliable discrete clusters in different regions of the olfactory cortex. Within the anterior pyriform cortex, the use of the two olfactory receptor promoters revealed different clusters that were partly overlapping but clearly distinct. A certain overlap between more diffuse projections to higher olfactory centers may constitute the anatomical basis for crosstalk between information strands emanating from different odorant receptors. This characteristic is probably helpful for integrating multiple modules of olfactory information into a composite gestalt, specific for a particular scent made of numerous chemical compounds (Korsching 2002).

Synaptic Transmission at the First Olfactory Central Relay

The main output neurons of the olfactory bulb, the mitral cells, are located in a single lamina, the mitral cell layer (Figure 11.1). Their primary dendrite, extending vertically from the soma, contacts one glomerulus, where massive interactions with periglomerular interneurons and olfactory nerve terminals occur. Periglomerular interneurons are a heterogeneous group of neurons that include both GABAergic and dopaminergic subtypes. In contrast, mitral cell secondary dendrites radiate horizontally, up to 1 μm, to span the olfactory bulb almost entirely (Mori et al. 1983). In the external plexiform layer, they interact with inhibitory axonless interneurons, the granule cells, which constitute the largest cellular population within the bulb. The output neurons are engaged with this extensive local circuit through dendrodendritic synaptic contacts. This reciprocal circuit provides inhibition that forms the basis for a reliable, spatially

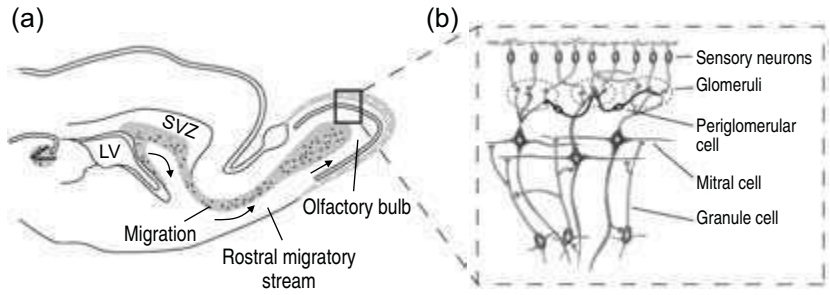

Figure 11.1 (a) The hippocampus and the subventricular zone (SVZ)–olfactory bulb pathway constitute the two neurogenic zones in the adult CNS. Arrows indicate the tangential migration of neuroblasts (dots) toward the olfactory bulb. Neurogenesis refers to the replacement of glutamatergic neurons in the hippocampus and of bulbar local interneurons in the olfactory bulb. Lateral ventricle (LV). (b) Wiring of the olfactory bulb. Each sensory neuron expresses only one of the 1000 odorant receptor genes, and the axons from all cells expressing that particular receptor converge onto one or a few glomeruli in the olfactory bulb. The nearly 2000 glomeruli in the rat olfactory bulb are spherical knots of neuropil, about 50–100 μm in diameter, which contain the incoming axons of sensory neurons and the apical dendrites of the main input-output neuron of the olfactory bulb, the mitral cell. Mitral axons leaving the olfactory bulb project widely to higher brain structures including the pyriform cortex, hippocampus, and amygdala. Processing of the olfactory message into the bulb occurs through two populations of interneurons: periglomerular cells and granule cells.

localized, recurrent inhibition of mitral cells (Rall et al. 1966). A mitral cell's synaptic depolarization, driven by the long-lasting excitatory input from the sensory neurons, triggers glutamate release by its dendrites and thus depolarizes interneuron dendrites and spines. This, in turn, elicits the release of GABA directly back onto the mitral cell. Thus, at the olfactory bulb level, the quality of the odor stimulus is first encoded by a specific combination of activated mitral cells, which depends on GABAergic inhibition. Since a single granule cell is believed to contact a number of output neurons (Shepherd et al. 2003), the reciprocal inhibitory synaptic connection contributes to the synchronization of mitral cell activity (Desmaisons et al. 1999; Kashiwadani et al. 1999; Lagier et al. 2004).

Following glomeruli activation, the network of output neurons and local interneurons adds a second combinatorial layer to the representation of olfactory information. Contacts mediated by bulbar output neurons and local interneurons may contribute to a global reformatting of odor representations in the form of a stimulus-dependent, temporal redistribution of activity across the olfactory bulb. According to this view, lateral inhibition is a means of sharpening odor representation by a neuronal population (see below). Depending on which sensory neuron afferents are stimulated and on which local interneuron connections become active as a result of this stimulation, different topologies of inhibitory circuits are expected to take form. Therefore, two combinatorial encoders

seem to take part in information processing. The first one consists of the olfactory receptor repertoire expressed by the sensory neuron ensemble that transduces receptor activation patterns into a glomerular "odor image" (Korsching 2002). The secondary encoder lies in the intricate interneuron network that extracts higher-order features from the odor images to convert them to timing relationships across the firing output neuron ensemble (Laurent 2002).

Coding Rules in the Olfactory Bulb

Sensory perception amounts to the deconstruction of the external world and the subsequent construction of its internal representation. To do this, sensory modalities rely on a specific coding that varies with the nature of external stimuli. A code is defined here as a set of rules by which information is transposed from one form to another. Regarding the sense of smell, sensory information is transduced first in the olfactory system by specialized receptor neurons located in the nasal epithelium. The olfactory bulb plays a central role in relaying olfactory information from the sensory organ to several central targets (Mombaerts 2001). In this structure, local circuits process the simple monophasic sensory signal conveyed by sensory neurons to convert it into a temporal code. There has been some controversy about the relative importance of spatial versus temporal patterns of odor-induced activity (reviewed in Friedrich 2002; Korsching 2002; Laurent 2002; Leon and Johnson 2003). Nevertheless, all data agree with nonstatic activity patterns in the bulb that evolve within a respiration cycle and from one cycle to the next. Odors elicit a well-organized pattern of activation in glomeruli. These activity patterns are distributed across the surface of the bulb and have to be considered as a function of time. Synchronizing mechanisms involved in temporal coding depend on the ability of different populations of GABAergic neurons to entrain the firing of principal neurons. Pioneering theoretical models have already suggested that synchronized activity (reflected by oscillatory rhythm) in the olfactory bulb (e.g., Rall and Shepherd 1968) is generated by a negative feedback loop between bulbar output neurons and local inhibitory interneurons.

From these studies, three assumptions can be drawn from the studies of information processing in the olfactory bulb. First, it has been proposed that neural synchronization of glomerular outputs may enhance the representation of a complex olfactory stimulus by integrating the different signal streams activated by the odor into a unified olfactory "image" at the level of the sensory cortex (Kashiwadani et al. 1999; Mori et al. 1999). Despite evidence supporting the use of time as a coding dimension in olfaction, much of the current thought on olfactory coding focuses on the importance of spatial patterns of activity. However, it seems likely that olfactory information is coded both in space and time (Friedrich and Stopfer 2001). The combination of spatial coding and correlated spike activity may, during odor stimulation, synchronize the spike responses of

the output neurons and the underlying process may be much more dynamic than previously thought. Second, the activation of neuronal assemblies in the bulb acts to modulate groups of neurons in the forebrain, which must bring into play some olfactory signal decoding mechanism. According to Mori et al. (1999), the olfactory forebrain uses coincidence detector neurons to identify the various activity patterns observed in the bulb. This model proposes that synchronized oscillations are a means by which signals from different olfactory neurons are bound in time in order to be detected. Finally, the neurons in the olfactory bulb that relay activity to the rest of the brain interact extensively with each other, both directly and through a network of coupled interneurons. The relay neurons can engage in highly synchronous oscillatory activity, thereby forming assemblies that, as a whole, convey information about a particular odor blend. I have suggested that the continuous turnover of GABAergic interneurons plays an important role in adjusting the spatiotemporal process of the olfactory bulb to the level of complexity and novelty of odors frequently encountered by the animal (Lledo and Gheusi 2003).

BUILDING A NEURONAL NETWORK

The migration of postmitotic neurons from their site of origin to their final destination, where they make synaptic connections, is a fundamental cellular event essential for building large neuronal assemblies (Rakic 1990). The telencephalon is the most complex division of the brain. It is broadly subdivided into dorsal (pallial) and ventral (subpallial) domains. As illustrated in Figure 11.2, cell migration within the telencephalon can be roughly subdivided into two categories: radial and nonradial.

The substrate for radial migration is the radial glial cell, which in the developing neocortex extends long, unbranched processes from the ventricular zone lining the lateral ventricle out to the cortical pial surface. All cortical neurons in primates, including humans, are generated during the first half of gestation within the proliferative zone lining the surface of the cerebral ventricle. After their final mitotic division, cortical neurons migrate away from their place of birth, toward the pial surface, where each successive generation passes the other and settles in an inside-out pattern within the cortical plate. Radially migrating cells give rise primarily to projection neurons that express the neurotransmitter glutamate (Figure 11.2; small arrows). In contrast, nonradially migrating cells that are generated in the ganglionic eminences give rise to a majority of the interneurons, and possibly other cell types such as oligodendrocytes, in the dorsal telencephalon (Figure 11.2; gray arrows). It is noteworthy that not only does the ganglionic eminence send interneurons dorsally to the overlying cortex but it also provides the subventricular zone (SVZ) of the postnatal lateral ventricle with proliferating neural stem cells (indicated by the small gray arrow in Figure 11.2). In this zone, they constitute a germinal region that persists into adulthood.

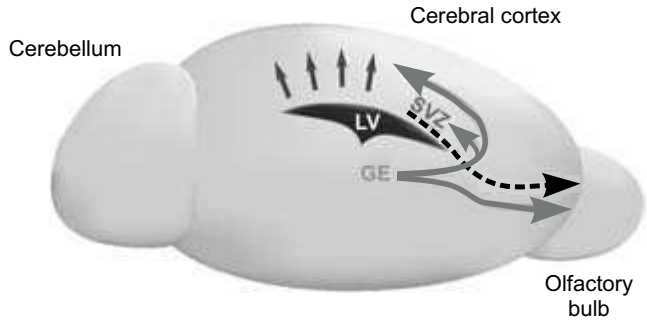

Figure 11.2 Migration of neuroblasts during embryogenesis and adulthood. Radial migration of cortical projecting neurons from the lateral ventricle (LV) to the developing cortex is indicated by small, black arrows. Gray arrows indicate tangential migration of neuroblasts from the ganglionic eminence (GE) to the neocortex, the olfactory bulb, and the subventricular zone (SVZ) of the lateral ventricle during embryogenesis. The dotted arrow indicates cell migration from the SVZ to the adult olfactory bulb.

The SVZ, once thought to provide the overlying cortex with glia late in development, is now also considered to constitute a steady supply of new interneurons for the adult olfactory bulb (dotted arrow in Figure 11.2).

Below I summarize how early work in this field has provided the framework for our current understanding of the different modes of interneuron migration in the adult olfactory bulb. I will also review the more recent findings that have dramatically reshaped the view of how newborn neurons are integrated within adult neuronal networks. At first glance, this neurogenesis may be seen as a putative response to the death and turnover of the primary olfactory receptor neurons that also occurs throughout life. I will demonstrate that, in fact, adult neurogenesis endows the bulb with unique properties.

DEVELOPMENT AND FUNCTION OF INTERNEURONS IN THE OLFACTORY BULB

In the embryo, bulbar interneurons are derived from neuronal precursor cells that migrate from the ganglionic eminence (Wichterle et al. 2001). Postnatally, they are derived from neuronal precursor cells that migrate in the rostral migratory stream (RMS) from the SVZ in the lateral ventricles of the forebrain (Temple and Alvarez-Buylla 1999). Within the SVZ, the neural precursor cells are considered to be stem cells since they proliferate and give rise to several different cell types (Temple and Alvarez-Buylla 1999). Unlike other immature neurons, precursor cells of the SVZ migrate tangentially without the aid of radial glial cells and continue dividing during their migration to populate the olfactory bulb, where they differentiate into local interneurons. Analysis of *in vitro* results gave rise to the current view that the adult SVZ generates stem cells capable of producing the three major cell types of the CNS (e.g., oligodendrocytes,

astrocytes, and neurons). When the neuroblasts reach the olfactory bulb, they migrate radially and, of those that survive, approximately 95% and 3% differentiate into GABAergic and dopaminergic interneurons, respectively. Below I discuss some of the recent findings that support functional implications for producing and integrating newborn neurons into the adult brain.

Although the ongoing neurogenesis and migration have been extensively documented, their functional consequences are still not clear. Since the largest neuronal population in the olfactory bulb consists of granule cells that outnumber the output neurons by at least 100:1 (Shepherd et al. 2003) and one granule cell contacts several mitral/tufted cells, and each mitral/tufted cell, in turn, contacts a large number of pyramidal cells from the pyriform cortex, the upstream location of the addition of transient newborn neurons in the olfactory bulb is ideal for amplifying the neurogenic effect within the entire olfactory pathway. I postulated that if newborn interneurons generated throughout life are necessary for olfaction, then modification of the migration processes (i.e., an alteration of the recruitment of newborn interneurons) affects olfactory processing. This hypothesis was tested using transgenic mice characterized by reduced adult neurogenesis. NCAM-mutant mice were used because they show reduced neuroblast migration. Quantifying the level of bulbar neurogenesis revealed a 40% reduction in the number of newborn interneurons in adult knockout mice. This impaired neurogenesis was accompanied by loss of odor discrimination (Gheusi et al. 2000), revealing a specific role for newborn interneurons in downstream coding of olfactory information. The assumption that a critical number of newborn GABAergic interneurons is necessary for normal odor discrimination was supported further using a different transgenic line in which $GABA_A$ receptor-mediated synaptic inhibition is altered (Nusser et al. 2001) as well as a theoretical approach (Cecchi et al. 2001). Together these data indicated that a critical level of inhibition, mediated by the activation of $GABA_A$ receptors localized on the secondary dendrites of bulbar principal neurons, is crucial for olfactory processing. In addition, since the olfactory bulb is also involved in consolidating processes associated with long-term odor memory, I proposed that changes in the number of GABAergic interneurons regulate both olfactory perception and memory.

To shed light on this issue, we investigated whether a change in the number of newborn bulbar interneurons alters olfactory memory. To challenge the level of neurogenesis, we raised animals in a complex olfactory environment (Rochefort et al. 2002). Exposure to an enriched surrounding has many positive effects on brain structure and function, including increased number of dendritic branches and spines, enlargement of synapses, increased glial numbers, and improved performance in tests of spatial memory (Rosenzweig and Bennett 1996). Exposure of adult rodents to increased environmental complexity induces neurogenesis in the hippocampus (Kempermann et al. 1998). We investigated whether an odor-enriched environment increases the number of newly formed interneurons

in the olfactory bulb and, if so, whether learning or memory is modified. We found that enriched mice produce twice as many newborn interneurons. This increase is specific to the olfactory bulb since the second neurogenic zone, the dentate gyrus of the adult hippocampus, remained unaltered. In addition, animals showing higher neurogenesis retain learned olfactory information for longer periods of time than controls (Rochefort et al. 2002). In particular, enriched animals are able to recognize familiar odors in a more durable and stronger way (i.e., they express a high resistance to retroactive interference) than animals raised in standard conditions. Although the potential consequences of an enriched environment on synaptic efficacy and/or mitral cell activity were not specifically explored, our results support the existence of a correlation between newborn bulbar interneurons population size and odor memory.

INTEGRATION OF NEWBORN NEURONS INTO ADULT CIRCUITS

It has been shown that neurogenesis in the adult as in the immature brain includes cell division, differentiation, and migration as well as synapse formation and programmed cell death. Until recently, however, only morphological (e.g., Kishi 1987) and immunohistochemical studies (e.g., Petreanu and Alvarez-Buylla 2002) supported the idea that cells generated in the SVZ in adulthood become true bulbar interneurons. Lack of direct functional evidence left open the possibility that the newly generated neurons never fully integrate into the bulbar network. In addition, whereas much work had been devoted to elucidating the birth and migration mechanisms of new SVZ neuroblasts, no information was available concerning their maturation. Such information is essential to appreciate how newly formed neurons become functionally integrated into adult neural networks. To obtain this type of information, we characterized the electrophysiological properties of newborn cells during their migration and differentiation using a replication-defective retrovirus that carries green fluorescent protein (GFP). GFP-labeled neurons were visualized in acute olfactory bulb slices prepared at different times following viral injection. We demonstrated that integration of functional neurons in adult neural networks is achieved through sequential steps in a highly regulated manner. These steps include proliferation of the neural stem cell, differentiation into an immature neuron, migration to the final location, growth of dendrites and formation of synapses with other neurons in the circuit, and ultimately maturation into a fully functional neuron (Carleton et al. 2003).

As early as 14 days after birth, some of the new neurons have reached the olfactory bulb and migrate radially to their final position. At this stage, they already display dendritic spines, which suggest that they are receiving synaptic inputs. Indeed, soon after new cells enter the layers of the olfactory bulb, they

express functional GABA and glutamate receptor channels and display voltage-dependent potassium currents typical of more mature neurons. Spontaneous synaptic activity then emerges soon after radial migration is completed. Interestingly, GABAergic and glutamatergic synapses impinging onto newborn neurons were established sequentially, GABAergic synapses being established first. In contrast to the sequential acquisition of synapses during embryogenesis, we found NMDA responses in neurons that were unable to fire and had started to receive $GABA_A$ and AMPA inputs. The rules that govern the incorporation of adult-generated neurons into mature neuronal circuits may thus differ from those described previously in the developing brain. In the adult brain, the correlated maturation of intrinsic electrical properties with synaptic activity of newborn cells may influence both maturation and integration processes and, thus, represents a mechanism by which neuronal activity may regulate neurogenesis.

Future studies will need to determine whether the newborn neurons make functional synapses with their downstream target neurons and release appropriate neurotransmitters in order to demonstrate unequivocally their integration into adult circuitries. Our data, however, indicate that adult-generated neurons, derived from neural stem cells in the SVZ, could physiologically grow into the olfactory bulb circuitry. Surprisingly, the spiking activity of newly generated neurons does not occur until late into the maturation process (Carleton et al. 2003). The delayed maturation of excitability may serve to prevent disruption of the adult, preexisting circuitry's function by the newborn cells. Further, this characteristic illustrates that maturation of adult-generated neurons does not recapitulate the embryogenesis, in part because of the high neuronal activity already occurring in the adult mature circuitry. In central sensory pathways, this activity results from external stimuli that lead to experience-dependent changes in anatomical connectivity, both during late development and in adulthood. Because these anatomical changes involve synaptic reorganization, understanding the maturation of synaptic transmission at newly formed synapses may provide important insight into how experience modifies already functioning neural circuits. The late occurrence of NMDA receptors and the maturation sequence of glutamatergic synapses we have reported may indicate unique maturation sequences adapted to experience-dependent maturation of adult neural circuits. Do newly generated bulbar neurons differ from older granule cells? Functionally, are there two types of granule cells: a larger population that is generated during development and early postnatal life and another one generated during adulthood? Answering these questions will clearly influence the ultimate interpretation of the role of adult neurogenesis. Finally, it should be kept in mind that only about half of the newly generated bulbar interneurons survive more than several days after having reached their mature state. We do not yet know if this survival time is sufficient for these cells to play a significant role in olfactory bulb function. Obviously, understanding the role of neuronal replacement in the adult brain implies that one must take into account that half of the newborn

neurons are transient and will be replaced through a process that has yet to be discovered.

PROPERTIES BROUGHT BY ADULT-GENERATED NEURONS

Although recent estimates suggest that the quantity of newly produced neurons in adulthood is much greater than previously thought, the rate of the adult neuronal production still remains lower than during development. As a result, if the functional properties brought by adult-generated neurons are similar to those brought by neurons generated in early life, then adult neurogenesis has to be considered insignificant (Nottebohm 2002). In contrast, if adult-generated neurons have unique properties that increase their impact relative to more mature neurons, then one can hypothesize that their constant integration into the functional circuitry has tremendous effects. Recent observations made both in the hippocampus and the olfactory bulb support the second hypothesis. Young granule cells in the adult dentate gyrus appear to exhibit robust plasticity that, in contrast to mature granule cells, cannot be inhibited by GABA. These newborn neurons may respond preferentially to modulation by stress hormones. Learning has been shown to increase the number of new neurons in the hippocampus. Running increases both the number of new dentate gyrus cells and performance on a hippocampal-dependent task, whereas a decrease in the number of new granule neurons is correlated with impaired performance for such a task (reviewed in Kempermann 2002). Thus, it is possible that since new neurons are structurally plastic, they are highly susceptible to changes in the animal's environment and to different life experiences. This suggests that adult neurogenesis is functionally important since its result is a continual influx of neurons that are, at least temporarily, immature with unique physiological properties. As described above, bulbar interneurons play an essential role in shaping the olfactory information that reaches the olfactory cortex. Since adult-generated cells born in the SVZ differentiate exclusively into bulbar interneurons, they probably contribute to essential aspects of olfactory processing. Although experimental studies and modeling have already provided arguments in favor of a permissive function role for newborn granule cells in olfactory discrimination, a more general function remains to be characterized. It is noteworthy that the functional benefit derived from adult neurogenesis cannot be acute since it takes several weeks to generate a functionally integrated new interneuron. We have shown that newborn cells extend neurites within a couple of weeks after cell birth, and it is obvious that the new connections cannot constitute a response to the particular functional event that triggered neurogenesis since these connections will only be in place well after the triggering event is over. Thus, bulbar neurogenesis has to be considered more a long-term than a short-term adjustment of the bulbar circuitry to a higher level of experience governed by olfaction.

ADULT NEUROGENESIS: A NEURAL BASIS FOR EXPERIENCE-INDUCED PLASTICITY

It is common sense to state that mind and brain benefit from an "active" life; this is backed by several observations, such as those from epidemiological studies. It is also well established that both physical and intellectual activities have a positive influence on the incidence of neurodegenerative disorders and on cognitive decline. Because of continuous neurogenesis in this region of adult animals, the olfactory system provides a unique model for studying the role of sensory-driven activity and its benefits. In the olfactory bulb, the role of environmental-induced activity can be explored not only during the neonatal period, when refinement and stabilization of developing structures takes place, but also in adulthood, when a more delicate regulation occurs. Several studies have demonstrated that modified levels of activity do not affect proliferation and tangential migration of neuroblasts. However, other studies have shown that in adults, odor enrichment increases the number of newly generated granule cells, whereas odor deprivation elevates neuronal death in the olfactory bulb. This indicates that sensory-driven activity may play an important role in the survival of progenitors and/or in their guidance from their migratory stream to the bulb. This, in turn, would lead to great changes in the number of newborn interneurons and, therefore, alter the inhibitory–excitatory balance in the bulbar neuronal networks. Despite clear evidence that adult neurogenesis can be regulated, it should be kept in mind that most data on adult mammalian neurogenesis have been derived from laboratory animals kept under rather artificial conditions compared to their natural habitat. Even the odor-enriched environments of our experiments are arguably much deprived compared to wildlife conditions.

Remarkably, when experience-induced plasticity is triggered, the opposite in terms of neurogenesis is found in young and adult animals. Challenging the level of sensory activity in adults alters their olfactory memory, whereas sensory deprivation performed at early stages does not affect olfactory performance. Thus, changing the level of sensory inputs may trigger homeostatic compensatory mechanisms only in newborn animals. During development, sensory activity might be important for refinement and maturation of anatomical and physiological connections established in an activity-independent manner, thus playing an instructive role in neurogenesis. In contrast, sensory activity during adulthood might play an informative role for newborn interneurons arriving at the olfactory bulb; this would allow them to reach a proper site in the mature neuronal network. Supporting this hypothesis are observations indicating that almost half of adult-generated neurons die shortly after reaching the olfactory bulb (Petreanu and Alvarez-Buylla 2002). It is conceivable that an alteration in the inhibitory–excitatory balance during development might have a much more profound effect on animal survival than during adulthood. Alternatively, the inhibitory–excitatory balance may be modified by sensory activity in a different way from one life stage to another.

ETHOLOGICAL RELEVANCE OF ADULT NEUROGENESIS

Although it is clear that the adult olfactory bulb integrates neurons throughout life, the reason for this remains unclear. What purpose do new neurons have, and how can an existing functional neural network integrate and even actively recruit new neurons? Prevailing theories of cognition are based on the assumption that the adult brain is a stable network with regard to the number of neurons. According to this scheme, structural neural plasticity occurs only at the level of synapses, dendrites, and neurites. The demonstration of adult bulbar neurogenesis (i.e., the generation of new interneurons from resident neuronal stem cells and their integration in the bulbar circuit) has called this assumption into question. In light of results on the activity-dependent regulation of adult bulbar neurogenesis, some conclusions regarding why and how new neurons may contribute to bulbar function can be made. I suggest that neurogenesis does not simply add neurons to enhance memory, but that it also strategically inserts newborn cells with unique properties. The multiple stages in the maturation of new neurons might serve as various substrates for plasticity. The newly formed neurons could provide the olfactory bulb with a large repository of plastic cells to respond better to environmental changes and to different life experiences. For instance, new neurons could respond preferentially to the modulation mediated by hormones as well as to learning and other forms of experience-induced activity. These aspects have been well documented in different biologically relevant models, which I describe here briefly.

Female mice form a memory of their mating male's pheromones, a phenomenon known as the Bruce effect. This memory prevents the newly mated female from sustaining her pregnancy when exposed to pheromones of other males (Bruce 1959). Such a memory is formed during mating and requires a period of about 3 to 4 hours of exposure to the male's pheromones. With a microdialysis approach, Brennan and colleagues (1995) explored the level changes of a range of neurotransmitters occurring when female mice form a memory of their stud male. In the accessory olfactory bulb, the main changes are a significant increase in noradrenaline release during mating and a decrease in the glutamate/GABA ratio following memory formation in response to the mating male's pheromones. The increase in GABA release illustrates a higher inhibitory tone at the reciprocal synapses. Brennan et al. interpreted such a mechanism as a way of reducing the excitation from mitral cells, which mediate the input from the mating male's pheromones, in order to prevent a pregnancy block. In contrast, exposure to a different male would activate a different subpopulation of mitral cells, not previously submitted to an increased inhibitory control, thus eliciting central pregnancy blocking mechanisms. These processes do not only occur during formation of a memory of the mating male's pheromones. Similar events have been found in the olfactory bulb of ewes when they form a specific ability to

recognize their own lamb and reject alien ones that attempt to suckle (Poindron and Levy 1990). After parturition, in response to odors from her own lamb but not to odors from alien lambs, an increase in glutamate and GABA release occurs in the ewe's olfactory bulb (Kendrick et al. 1992). Once the ewe has established a selective bond with its lamb, the ratio of glutamate to GABA is decreased in response to the lamb's odor, suggesting, once again, the existence of changes in the gain of reciprocal synapses between mitral and granule cells.

These two examples illustrate changes in the inhibitory–excitatory balance in the olfactory bulb occurring during the establishment of specific social preferences. In addition, similar changes have recently been shown to occur in circumstances involving olfactory learning in a broader context. For instance, a conditioning procedure, in which adult mice were trained with odor added to clean wood shavings through which they dig to obtain a buried piece of sugar, has revealed interesting findings. Following conditioning, the presentation of the odor previously associated with the reward induced an increase in both glutamate and GABA levels in the olfactory bulb as well as a decrease in the balance existing between excitatory and inhibitory transmitters. In contrast, no change was reported when animals were exposed to a nonconditioning odor, indicating that the increase in inhibition only occurs for relevant odors. These findings emphasize the significance of changes in the inhibitory–excitatory ratio that occur in the olfactory bulb following odor learning. It is noteworthy that the neurochemistry of inhibition enhancement is reminiscent of the up-regulation of the permanent supply of bulbar interneurons that correlates with a stronger memory in odor-enriched mice. Together, these findings indicate that adult bulbar neurogenesis might be involved in many natural behaviors that yet remain to be explored.

CONCLUDING REMARKS

We still do not know why there are new neurons in the adult brain, but studies on the regulation of adult neurogenesis, as well as an increasing number aimed directly at elucidating the function of the new cells, have led us to two conclusions. First, bulbar neurogenesis is affected by relevant behavior and, in particular, behaviors specifically related to bulbar function. Second, changes in rate or extent of neurogenesis have an effect on subsequent behavior. Thus, although behavior can change the structure of the olfactory bulb, changes in structure can subsequently change, or at least affect, subsequent behaviors. The correlation between activity and adult neurogenesis suggests that new neurons are involved in a general aspect of bulbar function, most likely in sustaining the ability of the adult network to adapt information processing to relevant ethological needs.

The sense of smell has played essential roles during mammalian evolution. Odor representation is dynamic and highly complex, perhaps requiring a unique mechanism of plasticity. Neurogenesis, migration, and replacement of new

neurons in the bulb are likely to play a part in this adaptive mechanism. Since it takes time for new neurons to mature and become synaptically integrated, adult neurogenesis may contribute to slow, long-term adjustments of the bulbar circuitry rather than to fast and acute plastic changes. However, faster mechanisms may also take advantage of new neurons. Several new bulbar interneurons die within a month after having reached their mature state. Our results show that newborn granule cells are synaptically connected during this period of fast cell death. Selective elimination of neurons may allow for a rapid modification of the circuitry. The ability to mold the integration of new neurons and to eliminate continually older cells without depleting the neuronal population may bring into neuronal networks a degree of circuit adaptation unmatched by synaptic plasticity alone.

In this brief review, I have addressed two of the main problems regarding the function of new neurons in the adult olfactory bulb. Both are fundamental issues. First, I discussed the role of adult neurogenesis in the context of the olfactory bulb's function. Under the assumption that the bulb processes odor information before relaying it to the olfactory cortex, I hypothesize that adult neurogenesis allows the olfactory bulb to adjust appropriately the degree of processing. Second, the existence of a pool of juvenile neurons, which enables the system to adapt to future similar situations, raises the possibility that adult neurogenesis acts post hoc to provide a structural basis for brain plasticity. Overall, I believe that research on adult neurogenesis in the olfactory bulb is not only intrinsically interesting, it provides a new avenue to the understanding of adult brain plasticity.

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REFERENCES

Bozza, T.C., and P. Mombaerts. 2001. Olfactory coding: Revealing intrinsic representations of odors. *Curr. Biol.* **11**:R687–690.

Brennan, P.A., K.M. Kendrick, and E.B. Keverne. 1995. Neurotransmitter release in the accessory olfactory bulb during and after the formation of an olfactory memory in mice. *Neuroscience* **69**:1075–1086.

Bruce, H.M. 1959. An exteroceptive block to pregnancy in the mouse. *Nature* **184**:105.

Carleton, A., L.T. Petreanu, R. Lansford, A. Alvarez-Buylla, and P.-M. Lledo. 2003. Becoming a new neuron in the adult olfactory bulb. *Nat. Neurosci.* **6**:507–518.

Cecchi, G.A., L.T. Petreanu, A. Alvarez-Buylla, and M.O. Magnasco. 2001. Unsupervised learning in a model of adult neurogenesis. *J. Comp. Neurosci.* **11**:175–182.

Desmaisons, D., J.D. Vincent, and P.-M. Lledo. 1999. Control of action potential timing by intrinsic subthreshold oscillations in olfactory bulb output neurons. *J. Neurosci.* **19**:10,727–10,737.

Friedrich, R.W. 2002. Real time odor representations. *TINS* **25**:487–489.
Friedrich, R.W., and M. Stopfer. 2001. Recent dynamics in olfactory population coding. *Curr. Opin. Neurobiol.* **11**:468–474.
Gheusi, G., H. Cremer, H. McLean et al. 2000. Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. *PNAS* **97**:1823–1828.
Kashiwadani, H., Y.F. Sasaki, N. Uchida, and K. Mori. 1999. Synchronized oscillatory discharges of mitral/tufted cells with different molecular receptive ranges in the rabbit olfactory bulb. *J. Neurophysiol.* **82**:1786–1792.
Kempermann, G. 2002. Why new neurons? Possible functions for adult hippocampal neurogenesis. *J. Neurosci.* **22**:635–638.
Kempermann, G., H.G. Kuhn, and F.H. Gage. 1998. Experience-induced neurogenesis in the senescent dentate gyrus. *J. Neurosci.* **18**:3206–3212.
Kendrick, K.M., F. Levy, and E.B. Keverne. 1992. Changes in the sensory processing of olfactory signals induced by birth in sheep. *Science* **256**:833–836.
Kishi, K. 1987. Golgi studies on the development of granule cells of the rat olfactory bulb with reference to migration in the subependymal layer. *J. Comp. Neurol.* **258**: 112–124.
Korsching, S. 2002. Olfactory maps and odor images. *Curr. Opin. Neurobiol.* **12**: 387–392.
Lagier, S., A. Carleton, and P.-M. Lledo. 2004. Interplay between local GABAergic interneurons and relay neurons generates gamma oscillations in the rat olfactory bulb. *J. Neurosci.* **24**:4382–4392.
Laurent, G. 2002. Olfactory network dynamics and the coding of multidimensional signals. *Nat. Rev. Neurosci.* **3**:884–895.
Leon, M., and B.A. Johnson. 2003. Olfactory coding in the mammalian olfactory bulb. *Brain Res. Rev.* **42**:23–32.
Lledo, P.-M., and G. Gheusi. 2003. Olfactory processing in a changing brain. *Neuroreport* **14**:1655–1663.
Maccaferri, G., and J.C. Lacaille. 2003. Hippocampal interneuron classifications: Making things as simple as possible, not simpler. *TINS* **26**:564–571.
Mombaerts, P. 2001. How smell develops. *Nat. Neurosci.* **4**:1192–1198.
Mori, K., K. Kishi, and H. Ojima. 1983. Distribution of dendrites of mitral, displaced mitral, tufted, and granule cells in the rabbit olfactory bulb. *J. Comp. Neurol.* **219**: 339–355.
Mori, K., H. Nagao, and Y. Yoshihara. 1999. The olfactory bulb: Coding and processing of odor molecule information. *Science* **286**:711–715.
Nottebohm, F. 2002. Why are some neurons replaced in adult brain? *J. Neurosci.* **22**:624–628.
Nusser, Z., L.M. Kay, G. Laurent, G.E. Homanics, and I. Mody. 2001. Disruption of $GABA_A$ receptors on GABAergic interneurons leads to increased oscillatory power in the olfactory bulb network. *J. Neurophysiol.* **86**:2823–2833.
Petreanu, L., and A. Alvarez-Buylla. 2002. Maturation and death of adult-born olfactory bulb granule neurons: Role of olfaction. *J. Neurosci.* **22**:6106–6113.
Poindron, P., and F. Levy. 1990. Physiological, sensory, and experiential determinants of maternal behaviour in sheep. In: Mammalian Parenting, ed. N.A. Krasnegor and R.S. Bridges, pp. 133–156. New York: Oxford Univ. Press.
Rakic, P. 1990. Principles of neuronal cell migration. *Experientia* **46**:882–891.

Rall, W., and G.M. Shepherd. 1968. Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. *J. Neurophysiol.* **31**:884–915.

Rall, W., G.M. Shepherd, T.S. Reese, and M.W. Brightman. 1966. Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Exp. Neurol.* **14**:44–56.

Rochefort, C., G. Gheusi, J.D. Vincent, and P.-M. Lledo. 2002. Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. *J. Neurosci.* **22**:2679–2689.

Rosenzweig, M.R., and E.L. Bennett. 1996. Psychobiology of plasticity: Effects of training and experience on brain and behavior. *Behav. Brain Res.* **78**:57–65.

Shepherd, G.M., W.R. Chen, and C.A. Greer. 2003. Olfactory bulb. In: The Synaptic Organization of the Brain, 5 ed., ed. G.M. Shepherd, pp. 165–216. Oxford: Oxford Univ. Press.

Temple, S., and A. Alvarez-Buylla. 1999. Stem cells in the adult mammalian central nervous system. *Curr. Opin. Neurobiol.* **9**:135–141.

Wichterle, H., D.H. Turnbull, S. Nery, G. Fishell, and A. Alvarez-Buylla. 2001. *In utero* fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development* **128**:3759–3771.

12

Axonal Wiring through Odorant Receptors

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ABSTRACT

The mouse's nose contains 2000 populations of olfactory sensory neurons (OSNs), each expressing one allele of one of the 1000 odorant receptor (OR) genes. An OSN projects its single axon to a single glomerulus from an array of 1800 glomeruli in the olfactory bulb. Within the glomerulus, the OSN axon synapses with the dendrites of second-order neurons in the olfactory pathway. Axons of OSNs that express the same OR project to the same glomeruli, which are located at characteristic positions on the surface of the olfactory bulb. Gene-targeting experiments that manipulate the OR coding region have revealed that the expressed OR determines both the odorant response profile of the OSN and the innervation of a specific glomerulus. Thus for an OSN to contribute meaningfully to an olfactory microcircuit, it must choose one functional OR for expression, and its axon must innervate a glomerulus corresponding to that OR (Mombaerts 2001). The mechanisms of OR gene choice and axonal wiring are central to the functional organization of the mouse olfactory system; however, our understanding of these processes is just beginning to develop. This chapter reviews a model that we have recently developed for the formation of the glomerular array. This contextual model proposes that axons of OSNs expressing the same OR sort themselves out and coalesce into glomeruli by OR-mediated homophilic and heterophilic interactions. The model can account for the characteristic positions of the glomeruli.

INTRODUCTION

In the mouse, odorants are detected in the main olfactory epithelium by $\sim$1000 types of odorant receptors (ORs) (Buck and Axel 1991; Zhang et al. 2004; Mombaerts 2004a). Odorant receptors are G-protein-coupled receptors with a putative seven-transmembrane (7TM) domain structure. Odorant receptor proteins are enriched in the dendritic cilia of olfactory sensory neurons (OSNs) (Feinstein et al. 2004; Strotmann et al. 2004). Odorant receptors transduce odorant binding through a G-protein/cAMP pathway that results in OSN depolarization and increase of spiking frequency. Generally, one OR gene (Malnic et

al. 1999), and one allele of it (Chess et al. 1994), appears to be expressed at high levels by an individual OSN, but the evidence for the one receptor–one neuron rule is not conclusive (Mombaerts 2004b). An OR gene is expressed by a few thousand OSNs, which are scattered across a part of the olfactory epithelium in a seemingly random arrangement. The boundaries of OR gene expression were originally defined as four broad "zones" in the main olfactory epithelium (Ressler et al. 1993; Vassar et al. 1993). Detailed analysis of these boundaries, however, suggests that each OR gene may have its own, unique pattern of expression (Iwema et al. 2004; Miyamichi et al. 2005).

In mouse and rat, an OSN has a single, unbranched axon that innervates a single glomerulus (Klenoff and Greer 1998). The analysis of axonal wiring in the mouse olfactory system has been greatly facilitated by a gene-targeting strategy that we developed a decade ago (Mombaerts 1996). Homologous integration of histological marker genes, such as taulacZ or tauGFP, at OR loci through gene targeting enables the specific labeling of cell bodies and axons of OSNs expressing a given OR (Mombaerts et al. 1996). Such OSNs send convergent axonal projections to a small area within the medial and lateral halves of each olfactory bulb (Ressler et al. 1994; Vassar et al. 1994; Mombaerts et al. 1996). Within each half-bulb, axons coalesce during development usually into a single homogeneous glomerulus (Potter et al. 2001; Treloar et al. 2002), where they synapse with dendrites of second-order neurons, mitral and tufted cells.

In a further elaboration of this gene-targeting strategy, we discovered that ORs are a determinant of axonal wiring (Mombaerts et al. 1996). The evidence comes from "swap" or replacement experiments, in which the OR coding region of a "recipient" OR locus is replaced with the coding region of a "donor" OR through gene targeting. A consistent finding is that axons of OSNs expressing a donor OR from the recipient OR locus (referred to as the "donor OR → recipient OR" replacement) coalesce into novel, ectopic glomeruli that are distinct from both the donor OR glomeruli and the recipient OR glomeruli (Mombaerts et al. 1996; Wang et al. 1998; Bozza et al. 2002; Feinstein et al. 2004). Thus, the OR is a determinant of axonal wiring, but it is not the only one. Consistent with a role of ORs in axonal wiring, OR proteins have recently been shown to be expressed in axons and axon terminals (Barnea et al. 2004; Feinstein et al. 2004; Strotmann et al. 2004).

How could axonal wiring be controlled by ORs? It is unusual for 7TM proteins to exert such control. Three types of models have emerged over the years. First, positional information in the form of gradients or specific cues could determine the positions for each of the 1000 OR-specific glomeruli on the surface of the olfactory bulb. An embodiment of this idea is the hypothesis that the OR sequence dictates positioning along the anterior–posterior axis of the bulb (Wang et al. 1998). However, additional OR replacement experiments (Feinstein et al. 2004) have not supported this hypothesis, which was based on a relatively small data set. Second, the sorting of axons into glomeruli could

merely reflect correlated odorant-evoked activity. The role of activity-dependent mechanisms in the olfactory system is mixed and remains controversial (Reed 2003; Feinstein and Mombaerts 2004; Yu et al. 2004). It is debatable whether natural odors, which elicit complex patterns of OR activation, could have enough discriminatory power to sort out 1000 axonal populations with great precision and relatively quickly during development, particularly prenatally. Third, OR molecules may impart specificity to axons interacting with each other and drive the self-organization of axons into glomeruli (Feinstein and Mombaerts 2004; Feinstein et al. 2004). We proposed that OSN axons sort themselves out into glomeruli through sampling of other axons with their growth cones. Sorting would be mediated by homophilic and heterophilic interactions between protein complexes that contain ORs or OR fragments. Differential affinities among axonal populations would produce a particular arrangement of glomeruli in an individual. This arrangement is conserved, but not stereotyped, in individuals of the same or similar genetic background. The outcome of this sorting process is contextual: it depends on which axonal populations are present.

In this chapter we review the evidence supporting our contextual model of self-sorting. Can it explain how an array of glomeruli is formed at characteristic positions?

TARGETS IN THE BULB?

Our contextual self-sorting model questions an implicit notion that is prevalent in a certain literature about glomeruli in rodents. It is commonly assumed that glomeruli emerge from preexisting structures in the bulb that serve as "targets" for axons of OSNs expressing a given OR. The targets are believed to be statically present for the OSNs that are born continuously throughout the lifetime of the individual. The prevailing thinking is influenced by the notion of "targets," which is reflected in the common use of such terms as "glomerular targeting," "axon targeting," "target selection," "target innervation," and "convergence onto glomeruli."

The notion of "targets" contributes to a rigid view of a stereotyped spatial map of topographically invariant glomeruli; in turn, the notion is enhanced by this view. If the positions of glomeruli are viewed as fixed and invariant among individuals, it is intuitive to regard these positions as specified by "targets" in the bulb. It is then logical to look for molecules with interesting expression patterns in the bulb. This avenue of research, however, has yielded fragmentary data and has been inconclusive as well as unconvincing.

What if there were no "targets" in the bulb? Can the establishment of an array of glomeruli at characteristic positions be explained without an elaborate system of positional information that would dictate the precise positions of 1800

glomeruli? For this alternative view, it is imperative to acknowledge the considerable degree of positional variability in glomerular positions. We have termed these variations "local permutations" (Strotmann et al. 2000). This variability can be appreciated by a careful examination of absolute or relative positions of specific glomeruli in the olfactory bulb, particularly by comparing both bulbs of the same mouse (Figure 12.1) (Royal and Key 1999; Schaefer et al. 2001; Strotmann et al. 2000; Feinstein and Mombaerts 2004). The extent of the local permutations is also obvious from the patterns of activated glomeruli upon odorant exposure, as revealed, for instance, by intrinsic signal imaging (Belluscio and Katz 2001) or imaging of the synaptic activity maker synaptopHluorin (Bozza et al. 2004). We question the portrayal of the glomerular array as a stereotyped map with topographically invariant positions.

Global studies of glomerular development in rat have not revealed any structural elements that predate the formation of glomeruli and to which axons would be attracted (Bailey et al. 1999; Treloar et al. 1999). To the contrary, these studies suggest that the formation of glomeruli is driven by OSN axons, not by the other cellular participants that could serve as "targets" for OSN axons (glia, interneurons, second-order neurons). An indication of "targets" has not emerged either from the studies of the development of specific glomeruli in mice generated by our gene-targeting strategy: the P2 glomeruli (Royal and Key 1999); the M71 and M72 glomeruli (Potter et al. 2001; Zou et al. 2004); and the OR37A, OR37B, and OR37C glomeruli (Conzelmann et al. 2001).

Our contextual model of self-sorting holds the alternative view that axons do not *converge onto* a glomerulus (implying a target), but *coalesce into* a glomerulus.

THE ODORANT RECEPTOR SEQUENCE IS THE PRIMARY DETERMINANT OF AXONAL IDENTITY

Our model has been largely derived from the analysis of gene-targeted mutations in the OR gene duo, M71 and M72 (Feinstein and Mombaerts 2004; Feinstein et al. 2004). The observations and our interpretation are summarized here.

M71 and M72 are expressed in the dorsal epithelium of the mouse, and axons of M71- or M72-expressing OSNs project to glomeruli in the dorsal bulb. The coding regions from the 129 mouse strain encode polypeptides with 96% amino acid identity; 11 of 309 amino acids are different. Gene-targeted mice were constructed in which M71 or M72 is co-expressed with the histological marker taulacZ or tauGFP. On average there are 1.2 glomeruli for each M71 and M72 per half-bulb; this means that $\sim$20% of half-bulbs have two M71 or two M72 glomeruli (Figure 12.1c, d). The M71 and M72 glomeruli are distinct: there is no cross-innervation of axons.

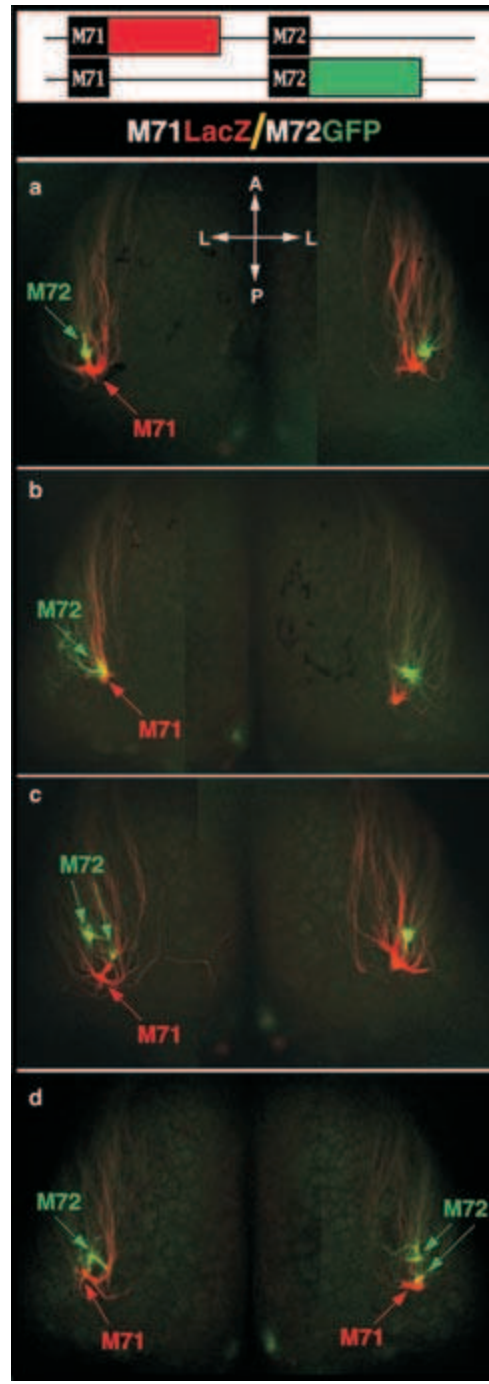

Figure 12.1 Positions for OR-specific glomeruli are characteristic but not stereotyped. Dorsal wholemount view of both olfactory bulbs of four mice heterozygous for M71-IRES-taulacZ and heterozygous for M72-IRES-tauGFP. Mice were 38 days old. A, anterior; P, posterior; L, lateral. Arrows point to the center of the glomeruli. M71 axons and glomeruli were visualized by the red X-gal/FastRed Violet staining of β-galactosidase activity; M72 axons and glomeruli were visualized by the intrinsic green fluorescence of GFP. The lateral glomeruli are visible; the medial glomeruli are out of focus and too deep to be well visualized. (a) The "textbook" pattern: the M72 glomeruli are anterior and lateral to the M71 glomeruli. (b) The distance between the M71 and M72 glomeruli is much smaller in the left compared to the right bulb. (c) There are two M72 glomeruli in the left bulb. (d) The M72 glomerulus in the left bulb is medial instead of lateral to the M71 glomerulus; there are two M72 glomeruli in the right bulb.

We hypothesized that, in this closely controlled situation, the OR is the only determinant of axonal identity. We thus generated the replacements M72 → M71 and M71 → M72. In contrast to the four other OR replacements reported previously (Mombaerts et al. 1996; Wang et al. 1998), the M71 and M72 coding regions are sufficient to re-specify glomerular identity when expressed from the respective locus. Therefore, the distinction between the M71 versus M72 glomerular identities must depend on the 11 amino acid differences.

To characterize the critical residues, we generated a series of targeted mutations at the M71 locus: the M71 OR backbone was modified into a more M72-like molecule through residue substitutions, creating a set of ten "hybrid" ORs (abbreviated Hyb). The axonal projections were analyzed in an extensive set of crosses between gene-targeted mouse strains. These crosses revealed that glomeruli are not necessarily homogeneous structures, but can exhibit various phenotypes that are dependent on the other axonal populations that are present. For instance, Hyb A axons are equivalent to M71 axons (the two types of axons co-converge and co-mingle); further, M71 axons are equivalent to M71 → M72 axons; but Hyb A axons are not equivalent to M71 → M72 axons. Thus, context matters. These glomerular phenotypes ranged between complete segregation into distinct glomeruli (as is the case for M71 and M72), and co-convergence to and co-mingling within the same glomeruli. Glomerular phenotypes can be intermediary: segregation into distinct glomeruli with reciprocal exchange of axons; co-convergence with incomplete intraglomerular segregation or compartmentalization; and co-convergence with complete compartmentalization. The notion of "targets" is difficult to reconcile with such intermediary phenotypes.

We view the spectrum of glomerular phenotypes as a product of "axonal identity": as a relative property of axons that is manifested depending on what other axonal populations are present. Our analysis of 53 crosses between 16 alleles of M71 and M72 revealed that multiple amino acids influence axonal identity. These residues are primarily distributed within predicted transmembrane domains of the OR. Surprisingly, a single amino acid substitution (D205N) in transmembrane domain V results in novel glomeruli. We did not determine the minimum number of residues necessary to convert the M71 axonal identity completely into that of M72. Complete conversion could be achieved by some combination of the seven amino acid substitutions that affect axonal identity; the strongest contribution coming from D205N, followed by V194I, A146V, V151M, and finally A157T, F28L, V36I. We examined only the naturally occurring amino acid differences between M71 and M72 in one mouse strain, 129. Axonal identity could be affected by many more residue substitutions throughout the OR molecule.

Could these glomerular phenotypes be secondary to similarities and dissimilarities between odorant response patterns? This is unlikely, because experiments that block odorant-evoked activity have no impact on the glomerular phenotypes (Feinstein and Mombaerts 2004).

NATURALLY OCCURRING POLYMORPHISMS IN ODORANT RECEPTORS ARE RELEVANT

We complemented our analysis of M71/M72 hybrids by studying the effects of amino acid polymorphisms on axonal identity for the ORs P2, P3, P4, M50, and M72 between the inbred mouse strains 129 and C57BL/6.

Two amino acid differences in M50 cause complete segregation of axons into distinct glomeruli in mice heterozygous for the 129 and C57BL/6 alleles. A single amino acid difference in P2 causes partial segregation within glomeruli: axons co-converge but do not co-mingle. More modest effects are seen for polymorphisms in P4 and M72, with axons partially segregating within glomeruli. P3 has no amino acid differences between the two mouse strains, and consistently, axons co-mingle within the same glomeruli. M71 is a pseudogene in C57BL/6; therefore, this strain has no M71 OSNs and M71 glomeruli.

Thus, in all examined cases with naturally occurring amino acid polymorphisms, we observe glomerular phenotypes that are similar to those of the M71-M72 hybrid ORs. The polymorphisms tend to reside in transmembrane domains, as is the case for M71/M72. The functional significance of these polymorphisms for axonal identity, and possibly for odorant responsiveness, implies that polymorphic OR alleles should be treated as separate OR genes. These polymorphisms could have contributed to the emergence of mono-allelic expression of OR genes. Because normal mice are not inbred and their genomes have numerous polymorphisms at the 1000 OR loci, the relative positions and identity of glomeruli are likely to differ substantially among individuals in natural populations of outbred mice. Perhaps individual (non-inbred) mice (or rats, or humans) each have a unique configuration of glomeruli.

ODORANT RECEPTOR LEVEL AS A SECOND DETERMINANT OF AXONAL IDENTITY

The analysis of OR replacements and hybrids suggests that the OR amino acid sequence determines the core of axonal identity. The similarity or identity in amino acid sequence is, however, only in certain, closely controlled situations, sufficient to re-specify axonal identity, as revealed by the rerouting of axons to the donor OR glomeruli. There must be other determinants.

The level of OR expression is another determinant. This factor is more difficult to measure and control experimentally because of the lack of quantitative assays for OR protein levels. Nonetheless we have provided two types of evidence supporting a role for the level of OR protein. First, correlative analysis of mouse strains with OR-tags reveals a range of presumed levels of OR protein, based on the indirect readout of marker intensity. Our estimates are that the relative levels of P2 and M50 are three times greater than M71 and M72, which are two times greater than P4 and MOR23, which in turn are two times greater than

P3. Interestingly, in OR replacements that reroute axons to the donor OR glomeruli completely or partially, the expression level per cell is similar for donor and recipient OR; in the OR replacements that fail to do so, the difference is at least twofold (Feinstein and Mombaerts 2004).

A second piece of evidence comes from a drastic reduction of M71 protein level, which we engineered by introducing an internal ribosome entry site (IRES) upstream of the M71 coding region by gene targeting (Feinstein et al. 2004). It is well established that translation from an IRES is less efficient than from a conventional translation initiation site. This modified M71 allele is expressed in OSNs that normally choose this locus for expression, but at a much reduced protein level—we estimate by a factor of tenfold. We assume that the identical M71 amino acid sequence is produced from the M71 hypomorph. Strikingly, axons of OSNs that express this presumptive M71 hypomorph axons coalesce into homogeneous glomeruli, but they are novel and located far more anterior and ventral to the M71 glomeruli. The formation of novel glomeruli at ectopic positions suggests that OR protein level is a determinant of axonal identity. The same OR can define at least two axonal identities.

POSITIONAL CELL TYPE AS A THIRD DETERMINANT

Most OR genes were originally thought to be expressed in one of four zones, defined by *in situ* hybridization with a relatively small set of OR probes (Ressler et al. 1993; Vassar et al. 1993). Several studies have reported overlaps in OR expression patterns (Norlin et al. 2001; Feinstein et al. 2004). A recent reexamination indicates that each OR may be expressed in a unique pattern and questions the concept of four zones (Iwema et al. 2004; Miyamichi et al. 2005).

We observe that our M50 → P2 replacement is expressed more broadly, presumably across three of the conventional zones including the part of the epithelium that contains OSNs expressing the unmodified M50 gene (Feinstein and Mombaerts 2004). Most M50 → P2 axons project to two novel, dorsal glomeruli; a subpopulation projects to a third glomerulus, which is the endogenous M50 glomerulus. Our interpretation of these findings is that the olfactory epithelium is composed of several OSN cell types, determined by the position within the epithelium. Positional cell type would thus be another determinant of axonal identity: it can modulate the axonal identity of OSNs that express the same OR at the same protein level. The position of an OSN within the epithelium would reflect differences in, for instance, expression or levels of transcription factors or adhesion molecules. Importantly, an OR can be expressed in more than one positional cell type—perhaps with different frequencies.

The spatial constraints imposed by the positional cell type on the axonal projections can be assessed by comparing the positions of glomeruli that are formed when the M71 coding region is modified by gene targeting. The five 7TM replacements we reported at the M71 locus (Feinstein and Mombaerts 2004;

Feinstein et al. 2004) and the presumptive M71 hypomorph (Feinstein et al. 2004) produce novel glomeruli in a discrete domain of the dorsal bulb. When the M71 coding region is deleted (ΔM71) and replaced by GFP, we observe OSNs that express another functional OR and that project their axons diffusely to many glomeruli in the same domain of the dorsal bulb (Feinstein et al. 2004). In these six targeted M71 mutations, the positional cell type in which the modified M71 locus is chosen for expression, is likely constant; the remaining variables are the OR amino acid sequence and protein level. Similarly, five OR replacements at the P2 locus, which is expressed in a more ventral part of the epithelium, produce novel glomeruli in a domain of the ventral bulb, corresponding to the area innervated by ΔP2 axons (Mombaerts et al. 1996; Wang et al. 1998; Feinstein and Mombaerts 2004). The OR deletion experiments further suggest that axons with a particular positional cell type are not predetermined to project to a small "target" area on the bulb, but can project to glomeruli that are broadly distributed within a large domain, as revealed by the expression of divergent ORs. Importantly, there do not appear to be molecules intrinsic to a positional cell type (for instance, glycoproteins, cell adhesion molecules) that lead to coalescence into glomeruli independently of the expressed OR.

It is not known how many positional cell types exist, and whether they are always spatially segregated. There are likely to be at least five such cell types, corresponding to the four conventional zones (Ressler et al. 1993; Vassar et al. 1993) and a "patch" of the olfactory epithelium in which a group of OR genes is expressed (Strotmann et al. 2000). The spatial constraints on the axonal projections imposed by a positional cell type may be regulated by a single adhesion molecule that is expressed differentially.

ODORANT RECEPTOR MISEXPRESSION LEADS TO ECTOPIC GLOMERULI

In several cases where an OR transgene is misexpressed in the olfactory epithelium, novel, ectopic glomeruli are formed in the olfactory bulb (Vassalli et al. 2002; Nakatani et al. 2003; Rothman et al. 2005). Such ectopic expression and ectopic glomeruli are also observed with targeted mutations of sequence motifs in the M71 promoter (Rothman et al. 2005). Interestingly, in some cases axons of OSNs expressing the endogenous, normal OR counterpart project to these ectopic glomeruli (Vassalli et al. 2002; Rothman et al. 2005). In an extreme case, all axons of OSNs expressing the endogenous OR gene project to the ectopic glomeruli (Rothman et al. 2005).

In one interpretation, what appears to be rerouting reflects normal but infrequent expression of the endogenous OR gene in another positional cell type. The bulk of the choices of the endogenous OR gene would be in a particular positional cell type (say, A), such that enough axons are available to form and maintain a major glomerulus. Infrequently the endogenous OR gene would be

expressed in a distinct positional cell type (B) that is sufficiently different for axons to be precluded from projecting to the major glomerulus; there may not be enough of these axons to form and maintain a second, minor glomerulus. However, when a second, major glomerulus is formed ectopically by the coalescence of axons of a large number of OSNs of positional cell type B expressing an OR transgene or modified OR locus, the minor population of axons from endogenous B OSNs can maintain stable innervation of this ectopic glomerulus, and these OSNs now survive.

THE CONTEXTUAL MODEL OF SELF-SORTING

We propose that sorting of OSN axons into glomeruli is based on OR-dependent homophilic and heterophilic interactions between growth cones extending on axon shafts. We do not favor direct homophilic interactions. Direct interactions would most likely involve extracellular domains, but M71/M72 OR residues that affect axonal identity do not reside within extracellular domains and may not be accessible for direct homophilic interactions. We favor indirect homophilic interactions, not between ORs directly, but between OR-containing complexes. The OR protein or a fragment would present itself as a unique three-dimensional structure that is differentially accommodated by associated proteins within the plasma membrane of axons and growth cones. These associated proteins would create unique complexes containing OR monomers, perhaps even dimers or multimers. Candidates for these associated proteins could be the novel molecules REEP and RTP that have been reported to promote surface expression of ORs in heterologous cells (Saito et al. 2004). Growth cones would reversibly sample axons for their similarity (homophilic), and such interactions would be stronger than happens for dissimilarity (heterophilic). This dynamic view of axonal projections is consistent with the morphology of the plexus of fasciculated and defasciculated axons as they approach their glomeruli (Potter et al. 2001; Conzelmann et al. 2001; Treloar et al. 2002).

Three-dimensional structures can be exquisitely sensitive to amino acid changes. In particular, changing the charge of a residue within a transmembrane domain would have a strong impact on the structure. We speculate that the concentration of OR protein may create different multimers of the OR-complex, thereby creating quaternary codes, which could amplify differences in OR structure. Further axonal identities may arise if associated proteins are expressed differentially among positional cell types.

We postulate that axons and growth cones emerge from the epithelium with an axonal identity. Molecular cues in the bulb might crudely define a limited number of domains that demarcate where specific glomeruli can form. During development the first few axons that arrive at the bulb may sort out by heterophilic (repulsive) interactions, causing them to congregate into a relatively broad region until they coalesce into proto-glomeruli by homophilic

interactions. The early arrivers need not be "pioneers" in the sense that they are qualitatively different from the bulk of the axons. Newly added axons would project to the proto-glomeruli using the axonal scaffold that has been formed. Our contextual model of self-sorting implies that sorting of 1000–2000 populations of axons into glomeruli could be achieved without a highly diversified set of chemical cues, perhaps without any gradient cue. In this view, axons do not look for targets—they are the targets.

REPRODUCIBILITY OF THE GLOMERULAR ARRAY

Earlier we emphasized the need to acknowledge the variability of the glomerular array, which is best appreciated when relative positions of two glomeruli are compared between the two bulbs of the same mouse (Figure 12.1). Self-sorting is expected to be error prone and less precise than guidance by bulb-derived molecular cues. Nevertheless, the conservation of glomerular positions is impressive. How can a self-sorting mechanism result in a glomerular array that is so conserved among non-outbred mice? (Mice of a mixed 129 × C57BL/6 background, which are commonly used in gene-targeting experiments, would qualify as non-outbred.)

We hypothesize that in addition to OR sequence, OR level, and positional cell type, the temporal expression pattern is a critical determinant. Unfortunately and surprisingly, ignorance rules. Very little information is available about the onset of OR expression and the kinetics of the expansion of the population of OSNs expressing a particular OR gene in mouse; moreover, such data have not been related to the dynamics of glomerular development in a comparative fashion among OR genes. No information is available about the first arrival of axons of OSNs expressing a particular OR gene, and their behavior at the surface of the olfactory bulb. No time-lapse imaging system, *in vivo* or in explants, is available for mouse. A global study of glomerular development indicates that the development of glomeruli in the rostral part of the rat olfactory bulb is two to four days ahead of the caudal part, but substantial heterogeneity exists within a microregion (Bailey et al. 1999). P2 glomeruli form in late gestation (Royal and Key 1999), OR37 glomeruli soon after birth (Conzelmann et al. 2001), and M71/M72 glomeruli a little later (Potter et al. 2001). Thus, the glomerular array develops asynchronously. Axonal wiring could be highly dependent on this asynchronous development; a glomerular array that develops synchronously may be much more difficult to wire.

We speculate that, in addition to OR sequence, OR protein level, and positional cell types, glomerular positions could be the outcome of when, where, and how often a particular OR gene is expressed before and during glomerular formation. A group of early-born and early-arriving axons may self-sort and coalesce into a proto-glomerulus at the first available position in the bulb that is appropriate for that positional cell type (say, extremely dorsally). Axons of another

OR expressed by the same positional cell type may achieve a threshold number for proto-glomerular formation slightly later and coalesce at the next available position in the extreme dorsal domain of the bulb. Axons may coalesce initially into several proto-glomeruli, which may not be homogeneous, and subsequent remodeling may be achieved by death of cells with inappropriately wired axons (Zou et al. 2004). We further suggest that axons may have different propensities to move on the surface of the bulb depending on the expressed ORs. This successive colonization of sites in domains of the olfactory bulb might produce a glomerular array that is characteristic (but not identical) among non- outbred mice. This hypothesis can be tested experimentally by systematically varying the number of OSNs that express a particular OR gene, in heterozygous mice or with transgenes.

Perhaps all that happens in some OR replacements where a novel, ectopic glomerulus is formed is that "when, where, and how often" has been changed. In this respect, ORs would, strictly speaking, not be "axon guidance molecules," but "identity" or "acquaintance molecules." The key to unraveling axonal wiring may lie in understanding the mechanisms that restrict the choice of expression among the repertoire of OR genes, in an OSN at a particular time in development at a particular position in the epithelium.

ODORANT RECEPTORS HAVE NOT EVOLVED A SPECIAL FUNCTION IN AXONAL WIRING

So far, none of the results discussed above, nor the contextual self-sorting model, attributes a specific function that ORs would have evolved in axonal wiring. Indeed, the β2 adrenergic receptor (β2AR) can substitute for an OR by every measure examined when expressed from the M71 locus by gene targeting (β2AR → M71 mutation).

β2AR has motifs in intracellular regions 2, 3, and 4 that are conserved among ORs and other 7TM receptors. β2AR can couple with G_{α}olf, implying functional similarity with ORs. β2AR shares 16% amino acid identity with M71 and 18% with the closest OR, which is comparable to 19% identity between the most divergent mouse ORs (Zhang et al. 2004). Remarkably, β2AR → M71 axons robustly form medial and lateral glomeruli at conserved and symmetrical positions, which are anterior and ventral to the M71 glomeruli. Like OR proteins, antibodies to β2AR reveal antigen in cilia, cell bodies, and axons of OSNs, as well as in the glomeruli. The β2AR → M71 glomeruli are innervated and functional by three criteria: they contain the synaptic protein synapsin; MAP2, a marker for dendrites of mitral cells; and the surrounding periglomerular cells express tyrosine hydroxylase, the expression of which is dependent on odorant-evoked activity. These axons innervate their glomeruli exclusively and homogeneously, suggesting that these glomeruli do not receive innervation from OSNs that express other ORs. If any of several other ORs were to be co-expressed with β2AR

→ M71, axons would likely innervate a variety of glomeruli, or not at all. It is difficult to imagine a mechanism that results in the co-expression of a specific OR or a specific set of ORs. Thus, it appears that β2AR is not co-expressed with any OR; however, direct proof is lacking (as is the case for other tagged OR genes!)

The β2AR is the best-studied G-protein-coupled receptor. By contrast, nearly no structure–function information is available for ORs, which are difficult to express in heterologous cells. The phenotype of the β2AR → M71 mutation holds the promise that information about structure–function relationships of β2AR can be applied to the wiring problem in the olfactory system.

AXONAL WIRING WITH 7TM RECEPTORS ELSEWHERE IN THE NERVOUS SYSTEM?

The OR protein is present in OSN axons, and ORs participate in axonal wiring. By extrapolation, 7TM receptors may provide identity to axons in other neural circuits. Genome-wide analyses of human and mouse have revealed 367 and 392 non-chemosensory 7TM receptor genes, of which 90% are expressed in the nervous system (Vassilatis et al. 2003). Admittedly, the convergence of thousands of like OSN axons provides a powerful assay to assess the function of ORs in axonal wiring; however, similar functions of 7TM receptors may be more difficult to recognize in other neurons. An important distinction is that the one 7TM receptor–one neuron rule is unlikely to be general. Instead, co-expression of distinct 7TM receptors could create a wide variety of combinatorial codes for neuronal connectivity. Such functions can be explored by the same gene-targeting strategy that we have developed for OR genes (Mombaerts et al. 1996; Feinstein and Mombaerts 2004).

REFERENCES

Bailey, M.S., A.C. Puche, and M.T. Shipley. 1999. Development of the olfactory bulb: Evidence for glia–neuron interactions in glomerular formation. *J. Comp. Neurol.* **415**:423–448.

Barnea, G., S. O'Donnell, F. Mancia et al. 2004. Odorant receptors on axon termini in the brain. *Science* **304**:1468.

Belluscio, L., and L.C. Katz. 2001. Symmetry, stereotypy, and topography of odorant representations in mouse olfactory bulbs. *J. Neurosci.* **21**:2113–2122.

Bozza, T., P. Feinstein, C. Zheng, and P. Mombaerts. 2002. Odorant receptor expression defines functional units in the mouse olfactory system. *J. Neurosci.* **22**:3033–3043.

Bozza, T., J.P. McGann, P. Mombaerts, and M. Wachowiak. 2004. *In vivo* imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* **42**:9–21.

Buck, L., and R. Axel. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* **65**:175–187.

Chess, A., I. Simon, H. Cedar, and R. Axel. 1994. Allelic inactivation regulates olfactory receptor gene expression. *Cell* **78**:823–834.

Conzelmann, S., O. Levai, H. Breer, and J. Strotmann. 2001. Brain targeting and glomerulus formation of two olfactory neuron populations expressing related receptor types. *Eur. J. Neurosci.* **14**:1623–1632.

Feinstein, P., T. Bozza, I. Rodriguez, A. Vassalli, and P. Mombaerts. 2004. Axon guidance to glomeruli by odorant receptors and the β2 adrenergic receptor. *Cell* **117**: 833–846.

Feinstein, P., and P. Mombaerts. 2004. A contextual model for axonal sorting into glomeruli in the mouse olfactory system. *Cell* **117**:817–831.

Iwema, C.L., H. Fang, D.B. Kurtz, S.L. Youngentob, and J.E. Schwob. 2004. Odorant receptor expression patterns are restored in lesion-recovered rat olfactory epithelium. *J. Neurosci.* **24**:356–369.

Klenoff, J.R., and C.A. Greer. 1998. Postnatal development of olfactory receptor cell axonal arbors. *J. Comp. Neurol.* **390**:256–267.

Malnic, B., J. Hirono, T. Sato, and L.B. Buck. 1999. Combinatorial receptor codes for odors. *Cell* **96**:713–723.

Miyamichi, K., S. Serizawa, H.M. Kimura, and H. Sakano. 2005. Continuous and overlapping expression domains of odorant receptor genes in the olfactory epithelieum determine the dorsal/ventral positioning of the glomeruli in the olfactory bulb. *J. Neurosci*. **25**:3586–3592.

Mombaerts, P. 1996. Targeting olfaction. *Curr. Opin. Neurobiol.* **6**:481–486.

Mombaerts, P. 2001. How smell develops. *Nat. Neurosci.* **4**:1192–1198.

Mombaerts, P. 2004a. Genes and ligands for odorant, vomeronasal, and taste receptors. *Nat. Rev. Neurosci.* **5**:263–278.

Mombaerts, P. 2004b. Odorant receptor gene choice in olfactory sensory neurons: The one receptor-one neuron hypothesis revisited. *Curr. Opin. Neurobiol.* **14**:31–36.

Mombaerts, P., F. Wang, C. Dulac et al. 1996. Visualizing an olfactory sensory map. *Cell* **87**:675–686.

Nakatani, H., S. Serizawa, M. Nakajima, T. Imai, and H. Sakano. 2003. Developmental elimination of ectopic projection sites for the transgenic OR gene that has lost zone specificity in the olfactory epithelium. *Eur. J. Neurosci.* **18**:2425–2432.

Norlin, E.M., M. Alenius, F. Gussing et al. 2001. Evidence for gradients of gene expression correlating with zonal topography of the olfactory sensory map. *Mol. Cell. Neurosci.* **18**:283–295.

Potter, S.M., C. Zheng, D.S. Koos et al. 2001. Structure and emergence of specific olfactory glomeruli in the mouse. *J. Neurosci.* **21**:9713–9723.

Reed, R.R. 2003. The contribution of signaling pathways to olfactory organization and development. *Curr. Opin. Neurobiol.* **13**:482–486.

Ressler, K.J., S.L. Sullivan, and L.B. Buck. 1993. A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* **73**:597–609.

Ressler, K.J., S.L. Sullivan, and L.B. Buck. 1994. Information coding in the olfactory system: Evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* **79**:1245–1255.

Rothman, A., P. Feinstein, J. Hirota, and P. Mombaerts. 2005. The promoter of the mouse odorant receptor gene M71. *Mol. Cell. Neurosci.* **28**:535–546.

Royal, S.J., and B. Key. 1999. Development of P2 olfactory glomeruli in P2-internal ribosome entry site-tau-LacZ transgenic mice. *J. Neurosci.* **19**:9856–9864.

Saito, H., M. Kubota, R.W. Roberts, Q. Chi, and H. Matsunami. 2004. RTP family members induce functional expression of mammalian odorant receptors. *Cell* **119**:679–691.

Schaefer, M.L., T.E. Finger, and D. Restrepo. 2001. Variability of position of the P2 glomerulus within a map of the mouse olfactory bulb. *J. Comp. Neurol.* **436**:351–362.

Strotmann, J., S. Conzelmann, A. Beck et al. 2000. Local permutations in the glomerular array of the mouse olfactory bulb. *J. Neurosci.* **20**:6927–6938.

Strotmann, J., O. Levai, J. Fleischer, K. Schwarzenbacher, and H. Breer. 2004. Olfactory receptor proteins in axonal processes of chemosensory neurons. *J. Neurosci.* **24**: 7754–7761.

Treloar, H.B., P. Feinstein, P. Mombaerts, and C.A Greer. 2002. Specificity of glomerular targeting by olfactory sensory axons. *J. Neurosci.* **22**:2469–2477.

Treloar, H.B., A.L. Purcell, and C.A. Greer. 1999. Glomerular formation in the developing rat olfactory bulb. *J. Comp. Neurol.* **413**:289–304.

Vassalli, A., A. Rothman, P. Feinstein, M. Zapotocky, and P. Mombaerts. 2002. Minigenes impart odorant receptor-specific axon guidance in the olfactory bulb. *Neuron* **35**:681–696.

Vassar, R., S.K. Chao, R. Sitcheran et al. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* **79**:981–991.

Vassar, R., J. Ngai, and R. Axel. 1993. Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* **74**:309–318.

Vassilatis, D.K. et al. 2003. The G protein-coupled receptor repertoires of human and mouse. *PNAS* **100**:4903–4908.

Wang, F., A. Nemes, M. Mendelsohn, and R. Axel. 1998. Odorant receptors govern the formation of a precise topographic map. *Cell* **93**:47–60.

Yu, C.R., J. Power, G. Barnea et al. 2004. Spontaneous neural activity is required for the establishment and maintenance of the olfactory sensory map. *Neuron* **42**:553–566.

Zhang, X., I. Rodriguez, P. Mombaerts, and S. Firestein. 2004. Odorant and vomeronasal receptor genes in two mouse genome assemblies. *Genomics* **83**:802–811.

Zou, D.J., P. Feinstein, A.L. Rivers et al. 2004. Postnatal refinement of peripheral olfactory projections. *Science* **304**:1976–1979.

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Topography and Dynamics of the Olfactory System

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ABSTRACT

The chemical senses—taste and smell—are evolutionarily the most ancient animal senses. They are characterized by a multidimensional and diverse stimulus space, consisting of many odorous molecules that cannot be classified along any small set of dimensions. Animals detect these odors with specialized olfactory sensory neurons that express one or a few ligand-binding odorant receptor (OR) proteins. Animals cope with the problem of recognizing an extremely large number of different odorants by programming a very large number of functionally different olfactory neurons. Odors activate these neurons and generate characteristic activity patterns across the population of these receptors, which are relayed to second-order olfactory neurons. The entire available raw information about the animal's olfactory environment is present in these patterns; however, olfactory information is further processed before it is relayed to higher-order brain centers. This chapter reviews the role played by individual neurons in microcircuits in the first olfactory synapse in modulating this information. How is information extracted about odor quality, odor concentration, and scent components? Identity, spatial, and temporal coding mechanisms are discussed.

INTRODUCTION

Microcircuits in the antennal lobe optimize olfactory coding. Such a statement clearly requires qualification. All animals with an advanced olfactory system have an "olfactory lobe" (the insect antennal lobe or the vertebrate olfactory bulb), in which axons from olfactory sensory neurons converge onto discrete, spherical structures—the olfactory glomeruli—local neurons make lateral connections, and output neurons relay the processed activity to higher-order

brain centers. "Olfactory coding" means transforming information about an olfactory stimulus into patterns of neuronal activity so that subsequent processing steps have access to that information. In that sense finding the "code" is equivalent to understanding the "language" of the brain. The code differs along the processing steps leading from the receptor neurons to a behavioral decision. For example, olfactory information is fully "encoded" within the sensory organs by the receptor neurons in the periphery, since all information about an olfactory stimulus that is available to the animal is in that activity pattern. Similarly, it is fully "encoded" within the olfactory lobe; the coding principles are, however, different and not yet fully understood. It is also fully "encoded" in the output neurons of the antennal lobe, most likely according to different coding principles. "Optimization" indicates that the code is improved along these steps. For example, coding is optimized when information relevant to the animal is enhanced with respect to less relevant content, when signal is increased over noise, when sensitivity is increased, and/or when the coding scheme is "reformatted" in a way that makes it easier and more reliable to decode so that information is better extracted. Such reformatting may allow information to be stored more economically and to be more suitable for memory formation, which would lead to an improved retrieval from memory. Such information could be the identity of an odor stimulus (an identification task), its concentration (a measurement along a continuous variable), or the identity of odor components in a mixture (an analytical task).

We consider the mechanisms that insect antennal lobes employ to optimize their coding capacity. Recent studies on insect olfaction have provided important insights into the function of the antennal lobe. Several controversies in the field remain to be settled, such as the significance of spatial versus temporal features, or reports that incoming activity patterns are not processed at this early stage (see below). Here we argue that the antennal lobe is actively involved in extracting relevant information from the sensory input, and that this process is accomplished by a set of several local neuron networks. We strengthen the notion of olfactory glomeruli as the functional units in this process. We propose specific working hypotheses for how odor identity and stimulus concentration are encoded, and how odor mixture analysis and high sensitivity are achieved.

BACKGROUND INFORMATION

We begin with a brief description of the neurons in the insect antennal lobe and then draw comparisons between different insect species as well as to vertebrates. We then review physiological properties. Discussion about the significance of morphological and physiological findings for olfactory coding is discussed thereafter.

Morphological Elements of the Insect Olfactory System

The Cells in the Insect Antennal Lobe

Odors are recognized by primary olfactory sensory neurons (OSNs), which are located on the insect antenna and express odorant receptor genes that encode the odorant receptors (ORs). These are seven transmembrane domain receptor proteins that interact with odor molecules and transduce odorant binding to cellular excitation. The organization of the insect olfactory system is shown in Figure 13.1, using the honeybee as an example. The OSNs send their axons to the olfactory neuropil, the insect antennal lobe (Figure 13.1a, b), which consists of discrete neuropil structures called olfactory glomeruli. Each OSN expresses a single OR gene, and all OSNs expressing the same OR converge onto a common glomerulus. A glomerulus collects OSNs of only one type. Thus, the glomerulus acts as a "collecting basket" of OSNs with similar odor response profiles. This correspondence has been shown for *Drosophila melanogaster* (Vosshall 2001) and is also assumed for other insect species, but has not yet been experimentally proven. However, a few cases of 1:2 and of 2:1 innervation ratios in *D. melanogaster* have been described as well. A glomerulus receives not only the input from OSNs, but contains a highly ordered synaptic organization including synaptic microcircuits among OSNs, local interneurons, and projection neurons. The cell bodies of local interneurons and projection neurons are located outside of the glomeruli, in the periphery of the antennal lobe. Local interneurons branch exclusively within the antennal lobe and, in honeybees, can be further divided into two classes (Figure 13.1c): homogeneous local interneurons globally innervate many glomeruli, whereas heterogeneous local interneurons innervate densely a single glomerulus and diffusely a few others (Fonta et al. 1993). Projection neurons represent the antennal lobe output neurons and relay the olfactory information to higher processing centers, such as the lateral protocerebrum and the mushroom bodies. Projection neurons are either uniglomerular neurons, and thus collect synaptic input in just one glomerulus, or multiglomerular neurons (Figure 13.1d). Uniglomerular projection neuron axons innervate both the lateral protocerebrum and the mushroom bodies, where they diverge onto many Kenyon cells, which in turn are read out by mushroom body extrinsic neurons. Multiglomerular projection neurons send their axons only to the lateral protocerebrum and around the α-lobe, bypassing the mushroom bodies. However, projection neurons in honeybees also have small branches at their exit points of the antennal lobe, which may form connections between cells outside their innervated glomeruli (Müller et al. 2002). The computational function of these lateral pathways is not yet understood.

Distinct classes of neurotransmitters mediate communication between different neuron types. OSNs release acetylcholine to excite local interneurons or projection neurons (Homberg and Müller 1999). Local interneurons are mainly inhibitory and use GABA and/or histamine to inhibit projection neurons, other

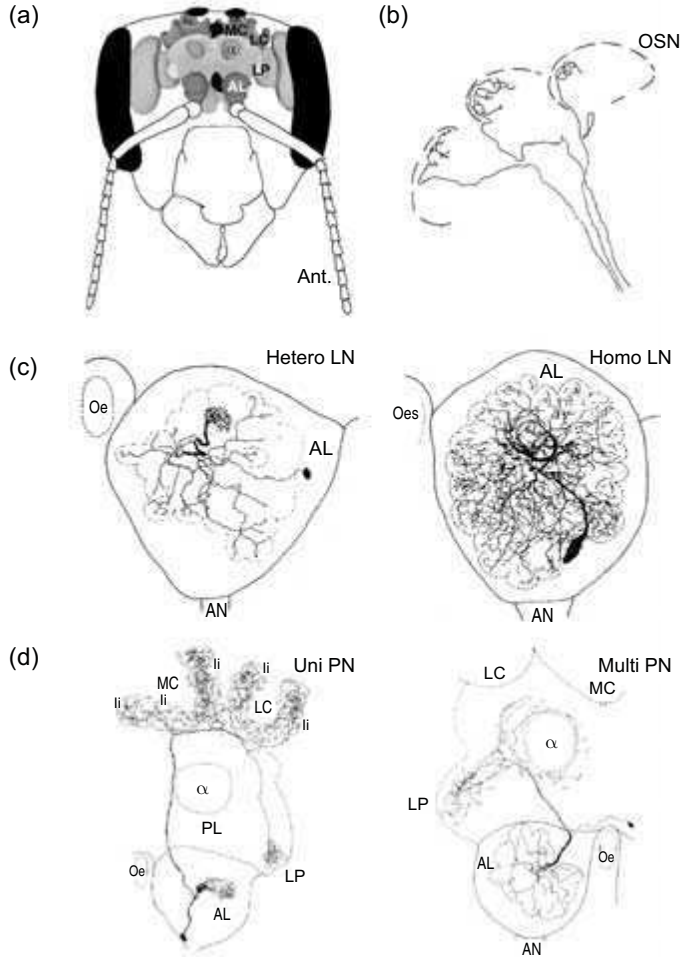

Figure 13.1 Morphology of olfactory neurons in the honeybee *Apis mellifera*. (a) Schematic view of the honeybee brain. The olfactory pathway leads from the antenna (Ant.) to the first olfactory neuropil, the antennal lobe (AL). The projection neurons (PNs) send their axons to higher processing centers: the lateral protocerebrum (LP) and the medial and lateral mushroom body (MB) calyx (MC and LC). α: α-lobe of the MB. (b) Reconstruction of three olfactory sensory neurons (OSNs). Each OSN innervates the rind of a single glomerulus in the AL (adapted from Brockmann and Brückner 1995). (c) Reconstruction of two different morphological types of local interneurons (LNs) in the honeybee AL. Heterogeneous LNs (hetero LNs) densely innervate a single glomerulus and sparsely a few others, while homogeneous LNs (homo LNs) diffusely innervate up to 100 glomeruli (adapted from Abel 1997). (d) Branching pattern of a uniglomerular (uni PN) and a multiglomerular projection neuron (multi PN) in the honeybee brain. Uniglomerular PNs collect the olfactory information of a single glomerulus in the AL and send their axons to the MB (medial and lateral calyx, MC, LC) and the LP. Multiglomerular PNs innervate many glomeruli and relay the information to the LP (adapted from Abel 1997). Oe = oesophagus; PL = posterior lobe.

local interneurons, or OSNs via a feedback loop. A subgroup of uniglomerular projection neurons uses acetylcholine, the transmitter of a second subgroup is as yet unknown, and at least some multiglomerular projection neurons release GABA.

Differences between Insect Species

Despite striking similarities among insects (and, in fact, between insects and vertebrates), there are many important differences (Figure 13.2): convergence ratios between OSNs and projection neurons, numbers of units, innervation logic of glomerular microcircuits, morphology of cells, and transmitters used.

For example, a single honeybee glomerulus is innervated on average by almost 400 OSNs (60,000 OSNs onto 160 glomeruli), 1000 local interneurons

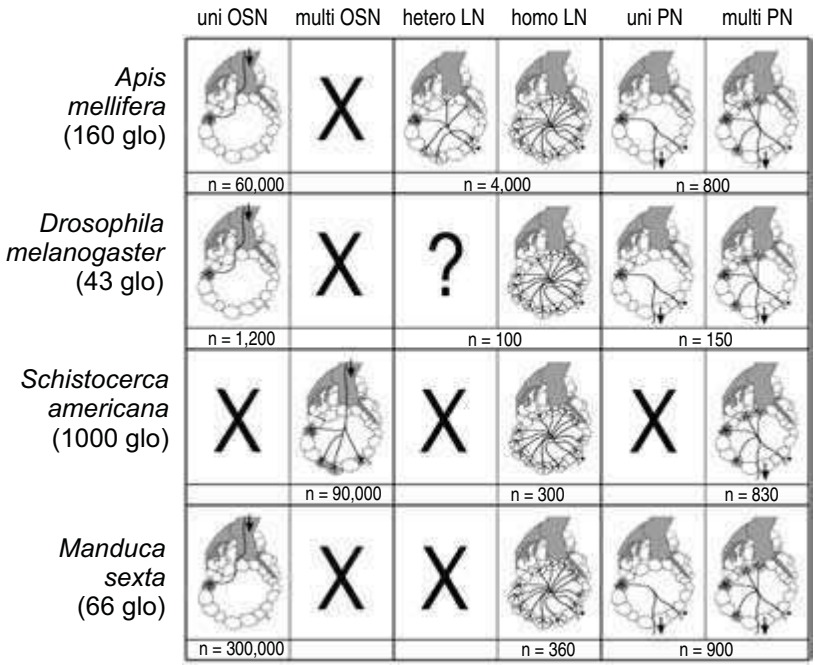

Figure 13.2 Olfactory sensory neurons (OSNs) in different insect species. The number of glomeruli (glo), neurons (n), innervation ratio, and neuronal morphology is species specific. If a particular cell type has never been described for a species, that entry is marked with an "X." Heterogeneous local interneurons (LNs) may be present in *Drosophila melanogaster*, but their morphology has not yet been described. Numbers are approximate and have been collected from a variety of references. In addition, if no separate count is available, only the sum of two cell types is given. In bees, most LNs are hetero LNs, but in flies that may be the other way round. Most projection neurons (PNs) are uniglomerular in bees, flies, and moths. It is unclear whether a more complex organization, per se, affords a more sophisticated behavioral spectrum.

(assuming a total of 4000 local interneurons and that each local interneuron innervates an average of 40 glomeruli), and 5 projection neurons (800 projection neurons in 160 glomeruli). The fruit fly *D. melanogaster* has many fewer OSNs and, therefore, a ratio between OSN and glomeruli of only 30:1. However, since most OSNs project into the antennal lobes of both sides, the average count of OSNs in each glomerulus is 50 in this species.

In contrast to other insects, locusts have only multiglomerular projection neurons. Similarly, individual OSN axons innervate several glomeruli. The glomerular groups innervated by projection neurons and OSNs do not coincide, suggesting that the multiglomerular organization is not a mere split of glomeruli into sub-glomeruli, but rather reflects a qualitatively different architecture. Indeed, locust antennal lobes consists of about 1000 "mini" glomeruli with different cytoarchitecture and innervation patterns. Therefore, in this species glomeruli either do not form functional units or the function of these units differs from that in other insects.

In addition to architectural differences, neurotransmitters also differ between insect species. For example, the occurrence of histaminergic local interneurons is not ubiquitous to insects. Apart from honeybees, only cockroaches and crickets possess histaminergic olfactory cells (Nässel 1999). The antennal lobes of moths, locusts, and dipteran flies lack histamine immunoreactivity. The species-specific function of histaminergic local interneurons, with regard to odor coding and processing, is still unknown and needs further investigation.

These species-specific differences show the obvious but often overlooked fact that generalizations among insect species have to be made with caution. Many features are shared, but each species is unique, and it may not always be possible to extrapolate observations made in one species to other species.

Comparison to the Vertebrate Olfactory Bulb

The olfactory bulb, which represents the analogue to the insect antennal lobe, displays important similarities in terms of anatomical and functional features (Figure 13.3). Both are spherical structures and consist of olfactory glomeruli. Current evidence suggests that, as in insects, OSNs expressing a given OR in mice project to a restricted number of topographically fixed glomeruli. Moreover, the vertebrate olfactory system has direct cellular counterparts for many of the cell types just discussed in insects: OSNs reside in the nasal epithelium and are morphologically similar to insect OSNs, mitral/tufted cells correspond to insect projection neurons, whereas granule cells and periglomerular cells are likely the functional relatives of insect local interneurons. Mitral/tufted cells are uniglomerular in rodents, but multiglomerular in zebra fish. Claiming a more detailed match (e.g., between heterogeneous local interneurons and homogeneous local interneurons with particular olfactory bulb interneurons) is inappropriate. One important anatomical difference between both systems is their layering: although all synapses of the antennal lobe are in the glomeruli, in the

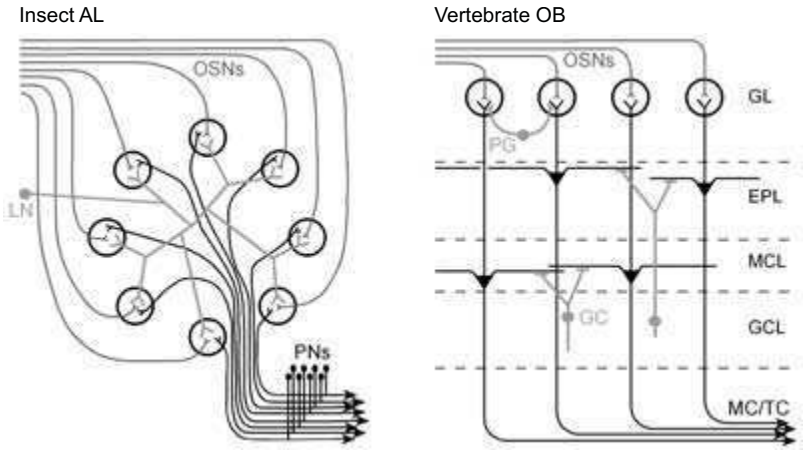

Figure 13.3 Organization of the insect antennal lobe (AL) versus the vertebrate olfactory bulb (OB). The olfactory neurons in the vertebrate OB represent counterparts of olfactory neurons in the insect AL. The OB, however, is organized in distinct layers, in which different processing steps take place, whereas in the AL, synaptic interactions only appear within a glomerulus. (OSN, olfactory sensory neuron; LN, local interneuron; PN, projection neuron; PG, periglomerular cell; GC, granule cell; MC, mitral cell; TC, tufted cell; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; GCL, granule cell layer). Adapted from Hildebrand and Shepherd (1997).

olfactory bulb, the glomerular layer is but one of several layers. In insects, projection neurons interact synaptically with local interneurons and OSNs almost exclusively within glomeruli. Their vertebrate counterparts show many synaptic contacts in deeper layers of the olfactory bulb distant from glomeruli.

Other Olfactory Systems

Specialized olfactory structures exist to process information about special stimuli, such as pheromones, which are used for intraspecific communication. These structures do not always follow the anatomical organization described above, which indicates that evolution has found a variety of solutions for olfactory coding. These structures are not considered here.

Physiological Responses to Odors

Responses in Olfactory Sensory Neurons

Odors elicit combinatorial across-OSN patterns of activity. Some odors may activate only one or a few OSNs, but most will activate several. As odor concentration is increased, activation is stronger, and more OSNs are recruited. The responses of OSNs have been characterized in a variety of species, with *D. melanogaster* being the best described system to date using single-sensilla recordings (de Bruyne et al. 2001).

Whether OSNs are narrowly or broadly tuned is a matter of vigorous debate. A system based on narrowly tuned OSNs is a labeled line system: each OSN only responds to a single substance, and thus when that OSN is active, the substance is in the environment. Such a system is very specialized, and in its extreme form, the number of odors that can be coded corresponds to the number of OSNs with different response profiles. In contrast, a system based on broadly tuned OSNs relies on combinatorial codes. Each odor activates many OSN types, and each OSN can be activated by many odors. It is only by analyzing the combination of active glomeruli that the animal can identify an odor. Many OSNs respond to a variety of substances at high concentration, but may be exquisitely sensitive to a few molecules at a concentration several orders of magnitude lower. Should we regard these as examples of a highly sensitive labeled line system, or rather as units in a combinatorial code? The answer lies in the ecology of the species: Even the most prominent cases of a labeled line (i.e., moth pheromone receptors) respond to chemicals other than the pheromone itself (indeed, some labs consistently use chemically more stable substitutes instead of the original pheromone component). However, such alien substances do not exist in the natural environment of these animals, and therefore a strong argument is rightly made for a labeled line system. For most other substances, however, animals are exposed to odorants over a large range of concentrations in their natural environment. A honeybee, for example, can smell an odor source over large distances (low concentration task), but can also recognize it when sitting on the flower (high concentration task). The olfactory system should therefore be capable of differentiating, for a given OSN, between the occurrence of a low-concentration best ligand or a high-concentration secondary ligand. This cannot be done on the basis of a single OSN response itself. It must be done through pattern analysis across several OSNs.

Most OSN responses are temporally structured. Some are inhibited by odors and show rebound excitation at the end of the stimulus; some fire for a long time irrespective of stimulus duration; others only fire for a very short time and even stop if the stimulus continues. Some of these effects are probably due to adaptation of the OSNs (tonic vs. phasic response properties). The consequence for the central nervous system is that it gets a temporally complex input from the OSNs. Whether these temporal structures are used for odor analysis and whether the periphery rather than the antennal lobe network may cause part of the temporal patterns observed within the antennal lobe is discussed below.

Responses in Local Interneurons

Most, or possibly all, local interneurons are inhibitory. Many are spontaneously active and respond to odors. In honeybees there is a prominent population of heterogeneous local interneurons that have distinct odor-response profiles comparable to uniglomerular projection neurons. In some cases the odors they responded to could be shown to correspond to the odors expected from the

functional atlas (Galizia and Kimmerle 2004). In *D. melanogaster*, homogeneous local interneurons show very broad response profiles, responding to most odors (Ng et al. 2002; Wilson et al. 2004). Local interneurons shape the responses in projection neurons in at least two ways: (a) with a very fast inhibition, mediated by GABA acting on ionotropic channels, and (b) with a slower timescale (Christensen et al. 1998). Studies with pharmacological antagonists have shown that the fast GABA-mediated currents cause synchrony among projection neuron spikes and odor-evoked oscillations in the range of 20–30 Hz. Local interneurons in honeybees, flies, and moths are spiking; in locusts they are non-spiking.

Responses in Projection Neurons

Responses in insect projection neurons have been measured at the single-cell level with electrophysiological techniques in a variety of species (Christensen et al. 1998; Hansson and Christensen 1999; Müller et al. 2002; Galizia and Kimmerle 2004) and with optical methods, by loading projection neurons with calcium sensitive dyes (in bees; Sachse and Galizia 2002, 2003) or by expressing activity-sensitive proteins in projection neurons only (in flies; Fiala et al. 2002; Ng et al. 2002; Wang et al. 2003). Comparing response profiles across many odors yielded somewhat contradictory results. In bees we found that the responses in projection neurons are modified with respect to the input, as estimated from bath applied dyes (Sachse and Galizia 2003), and shaped by the antennal lobe network, as shown by pharmacological manipulation (Sachse and Galizia 2002; Figure 13.4). This "reformatting" consists of the removal of weak glomeruli from the activity patterns. Therefore, whenever a glomerulus gave a strong response in the input, it also gave a strong response in the output. In contrast, other groups found in *D. melanogaster* that there was no difference between the OSN input and the uniglomerular projection neuron output in terms of molecular response profiles measured either by intracellular calcium increase (Wang et al. 2003) or by synaptic transmitter release (Ng et al. 2002). A recent study in *D. melanogaster*, however, contradicts these reports. Experiments using direct patch clamping of uniglomerular projection neurons and single-sensilla recordings of OSNs showed that uniglomerular projection neurons have broader response profiles than the OSNs that innervate the same glomerulus (Wilson et al. 2004). Because of the many differences in these studies, including different techniques, analysis methods, and experimental animals, we find that none of the studies described above is conclusive enough to draw firm conclusions about the logic of input–output relationships within olfactory glomeruli.

Antennal Lobe Output and Mushroom Body Input

What is the fate of across-projection neuron activity patterns on their way to the mushroom bodies? This question is not directly related to the antennal lobe, but

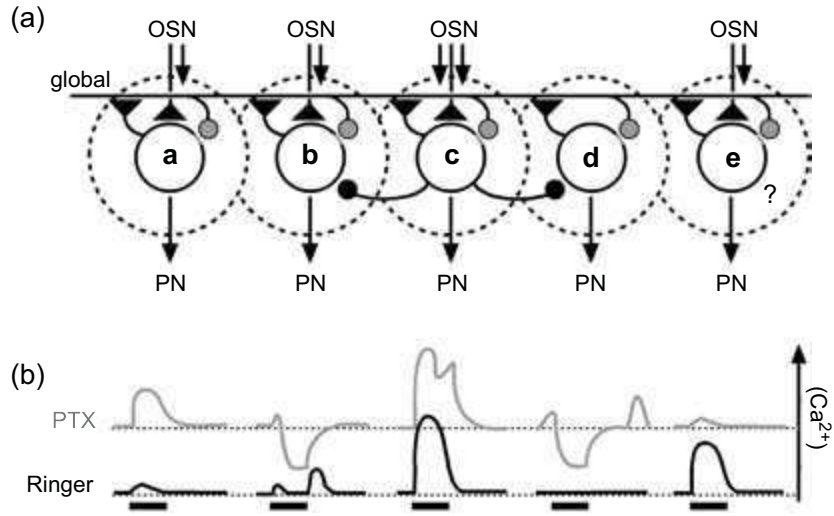

Figure 13.4 Two inhibitory networks shape the odor representation of projection neuron (PN) responses in the honeybee. (a) Model of the functional connectivity between olfactory glomeruli when stimulated with a particular odor. Glomerulus **c** gets strong OSN input and inhibits other glomeruli with picrotoxin (PTX)-insensitive synapses (black circles). Glomeruli **a**, **b**, and **e** get weak OSN input; **d** gets no OSN input for this odor. All glomeruli feed into a global, PTX-sensitive inhibitory network (gray circles). Thus, application of PTX leads to an increase of the PN response of weakly activated glomeruli **a** and a prolongation of glomeruli with a strong OSN input **c**. The tonic increase in intracellular calcium and spontaneous activity due to PTX leads to the calcium decrease becoming more visible; thus the inhibitory response of inhibited glomeruli is enhanced **b**, and inhibitory PN responses during odor stimulation are visible in previously nonresponding glomeruli **d**, in particular if they are spontaneously active shortly before the stimulus onset. The reduction of type **e** glomeruli following PTX application may be due to PN desynchronization. (b) Examples of time courses of PN calcium responses to odors: gray indicates superfusion with PTX; black depicts Ringer control. PTX leads to increased resting (Ca^{2+}) levels and changes the response properties, which can be categorized as five types of effects (a–e), corresponding to the glomeruli in the model. Figure adapted from Sachse and Galizia (2002).

it is still relevant, because understanding the next step may help us to decipher the output patterns. There is a massive divergence between projection neurons and the intrinsic neurons of the mushroom bodies, the Kenyon cells: in the honeybee, from 800 projection neurons onto 180,000 Kenyon cells. Although most projection neurons have a relatively broad response profile with various levels of activity strength, in Kenyon cells a large number of units encodes that same information with very few spikes, in almost binary fashion (each Kenyon cell is either active or not within each activity cycle), with only few units active at any time (Figure 13.5; Perez-Orive et al. 2002). Such logic strongly contrasts the antennal lobe, where each projection neuron covers a high dynamic range.

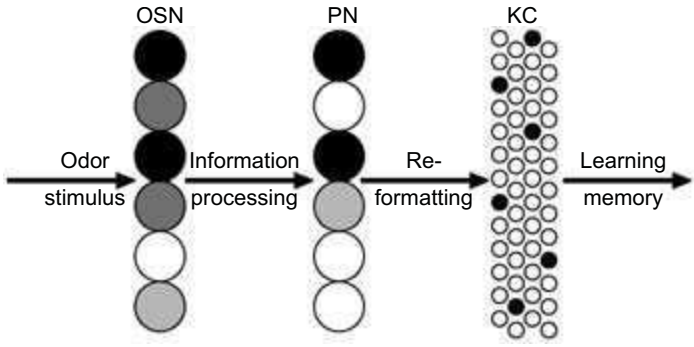

Figure 13.5 Processing levels in the antennal lobe (AL). Schematic diagram of the steps in olfactory coding. All information of an odor is necessarily coded in the combinatorial pattern of activity across receptor neurons (olfactory sensory neuron, OSN). This pattern is processed in the AL and modified into a new combinatorial pattern across projection neurons (PN). The pattern is reformatted in the mushroom body calyces onto a much larger number of Kenyon cells (KC). Note, however, the simplified nature of the figure; for example, olfactory memory traces are also found in the AL across PNs.

However, this transformation appears useful because it may facilitate the readout mechanism (Laurent 2002) and may allow more efficient learning (Heisenberg 2003).

THE OLFACTORY CODE

Coding at Sequential Levels

Looking at the olfactory system in a sequential fashion is a gross simplification, since several feedback channels influence the response patterns at all levels. Nevertheless, such an approach helps us to understand the essence of the code at each level, and all are part of "the" olfactory code. The levels to be considered here are OSNs (glomerular input), projection neurons (glomerular output), and Kenyon cells in the mushroom bodies (Figure 13.5). Olfactory sensory neurons produce a filtered and selective image of the chemical environment. It is trivial to state that all olfactory information available to the brain must be present in the combinatorial and temporally fluctuating activity patterns across the receptor neurons. It is likely that not all information present in the OSNs is processed in the antennal lobe, which would act as an additional filter for relevant information. The question is what and how information is extracted. Our view is that within the antennal lobe, the cellular network formed by OSNs, local interneurons, and projection neurons extracts information and creates an optimized odor representation, which is present at the output of the antennal lobe. The activity patterns across projection neurons are, again, combinatorial and temporally fluctuating (Figure 13.5). Finally, the massive numerical divergence

accomplished by the transition from projection neurons to Kenyon cells is associated with a further change in coding, which will not be addressed here (Laurent 2002; Heisenberg 2003).

Identity, Spatial, and Temporal Coding

Olfactory information is necessarily coded across the activity patterns of projection neurons, but how exactly this is done is still unclear. Much discourse has focused on the issue of whether coding is spatial, identity based, or temporal. Let us review these in sequence and ask whether there is any evidence of sufficiency (i.e., this coding could work alone) or necessity (i.e., without it, coding would not work) for each.

Identity Coding

When the identity of an active neuron is relevant, we speak of an identity code. In the olfactory system, this is certainly the case. A given odor elicits specific patterns of activity in glomeruli that can be predicted. For example, in the honeybee the response profile of 38 glomeruli (23% of all glomeruli) has already been described for 53 odors (Galizia and Menzel 2001). The molecular basis of this finding is becoming increasingly known in *Drosophila*, where a good match between receptor protein, glomerular identity, and projection neuron innervation pattern is found across individuals. Assume that we cut through the tract of axons connecting the antennal lobe to the mushroom bodies during odor stimulation: unless we know the identity of each axon, we will never be able to deduce the odor responsible for that pattern. Neither relative spatial position nor temporal patterns can help. On the other hand, if we know the activity pattern of olfactory glomeruli, with their identity, we can deduce the odor regardless of their relative spatial arrangement. Clearly, the olfactory code requires an identity component. However, even though the identity information may be sufficient, for an external observer, for the still limited odor/glomeruli space explored, we cannot exclude the possibility that the brain may need additional spatial and/or temporal features. Each insect glomerulus is innervated by 3–5 projection neurons, and in some cases these projection neurons leave the antennal lobe via different tracts. Whether these are redundant or have different properties is unknown. Electrophysiological recordings of honeybees' projection neurons suggest that projection neurons from different morphological tracts afford different processing properties (Müller et al. 2002). An identity code across glomeruli is necessary and potentially sufficient.

Spatial Coding

As pointed out by Laurent (1999), an identity code does not imply a spatial code. Is the spatial pattern of activity relevant for the code? Presumably, within the

axon bundle to the mushroom bodies, it is not. However, efficient processing within the antennal lobe may depend on the spatial position of olfactory glomeruli. This view has been strongly advocated for the mammalian olfactory bulb, where local neurons may create a center-surround inhibitory network (Aungst et al. 2003). In many brain areas, convenient spatial arrangement optimizes processing and/or total wiring length (e.g., in the visual system; Durbin and Mitchison 1990). Spatial arrangements also produce a topology: In the primary visual cortex, several representation maps are overlaid in a way that has often been shown to be optimal (a retinotopic map, an ocular-dominance map, an orientation and movement map, and a color-coding map are all superimposed in two dimensions). For a multidimensional olfactory world, two dimensions are not sufficient. Therefore, neighborhood relationships may be useful for subgroups of glomeruli but need not be a general rule. For example, in the honeybee, the neighboring glomeruli T1-33, T1-17, and T1-28 form a continuum in the representation of alcohols from 1-nonanol to 1-hexanol, with continuously decreasing carbon chain length (Galizia and Menzel 2001). Still, these odors also activate glomeruli that do not have neighbors with similar response profiles. A look at the morphology of local interneurons strengthens this point: Cell bodies are clustered together, and the main dendrite travels toward the center of the antennal lobe. From this position, all glomeruli are approximately equidistant. There is only little gain in innervating direct neighbors as compared to some more distant glomeruli in such a spherical arrangement. One step further along the processing path, the spatial arrangement of glomeruli in the antennal lobe may also be relevant by influencing the neighborhood relationships of projection neuron target areas in the mushroom bodies and lateral protocerebrum. Indeed, projection neurons with similar axon projection patterns in the lateral protocerebrum tend to receive input from neighboring glomeruli in *Drosophila*, suggesting that the organization of the lateral protocerebrum mirrors aspects of the organization in the antennal lobe (Marin et al. 2002; Tanaka et al. 2004). The functional relevance of such a map still needs to be proven.

In summary, while possibly used in subgroups of glomeruli, spatial position of insect glomeruli may generally be dictated more by developmental and/or genetic factors, rather than computational constraints. The spatial position of a glomerulus may even be a necessary component for accurate development of the antennal lobe. We note, however that these are two different aspects; if the identity of a neuron is developmentally characterized by its spatial position (e.g., along a gradient, or by molecular markers), then "space" is a necessary variable for a correct wiring of the network. In the fully developed system, this does not mean that "space" is used for computing the olfactory code. Therefore, from a computational point of view, spatial coding in the adult antennal lobe is not necessary, let alone sufficient.

Temporal Coding: Slow

Odor-evoked activity patterns evolve over time within one stimulus application, and projection neurons differ with respect to their temporal response properties (Müller et al. 2002). It is not clear whether part of this phenomenon already originates in the OSN responses, which also show complex dynamics in their activity (see above). Within the antennal lobe or olfactory bulb, this slow evolution ameliorates odor representation, leading to a clearer distinction of odors (Galizia et al. 2000; Friedrich and Laurent 2001). How much of this process can help the animal make better decisions? Among the peculiarities of the olfactory system is the recent finding that information about an odor stimulus does not increase with time, but is rather processed in fixed time units (Ditzen et al. 2003; Uchida and Mainen 2003). In these experiments, the time that an animal takes to reach a decision about a previously learned odor against a similar one was measured. Irrespective of odor similarity (and thus task difficulty), the time needed was constant: 690 ms for honeybees and about 200 ms for mice (this included the time needed for olfactory recognition as well as motor responses and physical displacement of the animal). Physiological studies of projection neuron responses show that 200–300 ms in locusts (Stopfer et al. 2003) and 400 ms in bees (Sachse and Galizia 2003) are needed to reach the most distinct odor classification in the antennal lobe, irrespective of odor concentration. Whether the brain reads the sequence of activities up to that point, or takes a snapshot of the optimized representation, remains to be addressed experimentally. The onset time of a stimulus is probably encoded by the homogeneous local interneurons and multiglomerular projection neurons—neurons that innervate most if not all glomeruli fairly uniformly. Any fluctuation in odor-driven activity that happens after that time span is not relevant for the animal in taking a decision regarding this stimulus. They may, however, still be relevant to the processing of future stimuli (e.g., for repetitive stimuli or aspects involved in memorizing an odor). Since we have already argued that the code has a necessary identity component, slow temporal patterns cannot be sufficient for olfactory coding. They may still be necessary, but as yet we do not have conclusive evidence.

Temporal Coding: Fast

Fast temporal encoding involves synchrony between spikes and their relationship with oscillations. Laurent (2002) has suggested a feedback loop within the mushroom bodies, formed by the input from the antennal lobes (the projection neurons), the Kenyon cells, and lateral horn inhibitory neurons. This loop would efficiently extract only those action potentials from the projection neuron ensemble code that are in phase with the oscillatory activity, (i.e., only these spikes transmit the information from the antennal lobe to the mushroom bodies). This process is relevant for the transition between projection neurons and Kenyon cells. The question arises, however, whether this mechanism implies that all

nonsynchronized action potentials in projection neurons are wasted; that is, whether they are not involved in coding the odor at all. Given that firing action potentials are among the most energy-intensive activities of the brain (Attwell and Laughlin 2001), such a waste would appear quite inefficient. Indeed, "surplus" spikes may not be wasted at all: Projection neurons make synaptic output within the antennal lobe, and this output is likely not to be filtered by a fast temporal constraint. Therefore, a mechanism that would extract only a subset of action potentials (which are synchronous with other action potentials) as having an effect in the mushroom bodies does not make the other action potentials a waste of energy. They would still influence olfactory coding within the antennal lobe, and possibly in the lateral protocerebrum. As a result, fast temporal spiking patterns may not be relevant for olfactory processing and optimization within the antennal lobe, even if they may be important outside the antennal lobe, in the reformatting of information between antennal lobe and mushroom body.

The pharmacological agent picrotoxin (PTX) blocks chloride channels such as $GABA_A$ receptors and thus partially removes the effect of local inhibitory networks. Under its influence, bees fail to discriminate two similar odors, but the distinction of two clearly dissimilar odors is not impaired (Stopfer et al. 1997). At the time of publication, the only known effect of PTX was that it disrupted fast oscillation, and consequently the experiment was taken as evidence that fast oscillation and/or synchrony of action potentials are a necessary component of accurate olfactory coding. We now know that PTX also affects the identity code (Figure 13.4). Some projection neurons that do not normally respond to an odor do so when PTX is applied; others stop responding in honeybees (Sachse and Galizia 2002) as well as in flies (Wilson et al. 2004). Therefore, the loss in olfactory discriminatory power observed under PTX may be a result of the suppressed synchronization or of the changed overall activity across projection neurons. To date, we have no experimental evidence to suggest that fast temporal components are necessary for coding odors, nor do we have evidence to the contrary.

Olfactory Glomeruli Extract Information

For almost every organism, it is essential to encode different aspects of an olfactory stimulus. This means that the quality of an odor should be coded separately from its intensity. Our visual system, for example, has developed circuits for extracting color information over a wide range of light intensities, so that we can recognize the color of a daisy in sunlight as well as in bright moonlight. Moreover, since odors usually occur in nature as complex blends, the olfactory system needs a specific code for each possible odor combination; it should also be able to analyze single components composing mixtures. Next we discuss theories regarding a multichannel readout strategy by the olfactory system to accomplish these complex requirements.

Signal-to-Noise Ratio versus Sensitivity

The antennal lobe must first extract meaningful signals from background noise. However, what constitutes in the antennal lobe the signal or noise? Odor-evoked activity patterns are the signal, while activity that is random is noise. It appears odd, then, to observe that projection neurons have very strong and random spontaneous activity, as shown in many electrophysiological recordings. Such a situation does not exist at all in the input. Although some OSNs are spontaneously active, this activity is not temporally complex. In calcium imaging studies, where only projection neurons were labeled, it was possible to observe frequent bouts of coactive glomerular patterns over long stretches of time (see examples at http://galizia.ucr.edu). An experimenter may not be able to distinguish individual events of spontaneous activity from an odor-evoked pattern. The antennal lobe thus appears to decrease the signal-to-noise ratio rather than increase it. What could be the evolutionary advantage for this?

We propose that this is the price for increasing the antennal lobe's sensitivity to weak odor concentrations. Metaphorically speaking, the antennal lobe is a loaded spring of always almost active projection neurons. On the cellular level, homogeneous local interneurons could accomplish such a task since they have access to all glomeruli and can thus inhibit them all. This feedback loop constantly keeps the projection neurons at firing threshold, so that minimal olfactory stimuli will already elicit an odor-evoked pattern. The mechanism for maintaining the set value at the threshold level is to probe it constantly, which is what we see as spontaneous activity. The cost is a loss in signal-to-noise ratio over time. At first sight, this mechanism may interfere with a reliable representation of odor concentration. However, this is not the case. What interferes with the representation of odor concentration is odor adaptation at the periphery, a phenomenon known from all sensory systems. The phasic response properties of OSNs ensure that the olfactory system always measures concentration changes rather than absolute concentrations. In the adapted state, the "loaded spring model" of the antennal lobe ensures that even small increases in odor concentration will lead to them being detected by the animal. The magnitude of this odor concentration increase (i.e., the strength of the olfactory stimulus) remains reliably coded in the response strength across olfactory glomeruli (see below).

There is a similar phenomenon in hearing. Acoustic sensory neurons have physiologically labile mechanical responses, including intensity-dependent nonlinear effects and spontaneous otoacoustic emissions (Hudspeth et al. 2000). These mechanisms greatly increase acoustic sensitivity. The spring model that we propose serves the same scope, even though not realized at the level of sensory neurons, but rather centrally with a fast neural network. The pendant of otoacoustic emissions in the auditory system are the spontaneous activity patterns across glomeruli in the olfactory system.

Is there any evidence for this model, apart from the observed spontaneous activity? We think there is currently none, and appropriate experiments should be

designed. However, there are two experimental findings that would indirectly support this notion, if we add a memory component to the "loaded spring model." One has been published by Stopfer and Laurent (1999): repeatedly giving an odor to a locust increased the signal-to-noise ratio of the resulting activity pattern in the projection neurons, a phenomenon interpreted as being the result of a form of memory within the antennal lobe. This memory may be a shaping of the "loading forces" in the "loaded springs" of the antennal lobe, so that activity corresponding to that odor would preferentially become suprathreshold. Another experiment was performed by R. Galan and M. Weidert (unpublished). In calcium imaging experiments in honeybees, they found that traces of a given odor persist in the patterns of spontaneous activity for several minutes, providing direct evidence of such reshaped "loading forces."

Odor Identity as Combinatorial Patterns

As noted above, an identity code with individual glomeruli as units is sufficient for odor identification from the activity patterns. Since the input pattern is contrast enhanced and optimized within the antennal lobe network, the output pattern is even more "powerful" in coding an enormous number of distinct activity patterns and thus olfactory stimuli. However, if odor quality is represented by the identity component of the odor responses, it requires concentration-invariant activity patterns over a wide range of odor concentration to ensure correct odor quality recognition. Indeed, calcium imaging responses of projection neurons in honeybees were qualitatively stable over a concentrations range of up to 4 log units (Sachse and Galizia 2003), with stronger odors increasing response intensity without changing the relative pattern across glomeruli. Interestingly, at the input level the activity patterns were quite affected by odor concentration. Thus odor quality coding and concentration invariance is probably achieved by the neural network within the antennal lobe. Heterogeneous local interneurons are most likely responsible for this process: They selectively innervate a limited number of glomeruli and can therefore optimize very specific contrasts of glomerular activity. The identity of an odor could thus be represented in the combination of activated glomeruli and could be relayed by uniglomerular projection neurons to higher processing centers.

The similarity between microcircuits in the retina and the olfactory bulb has been pointed out by Shepherd and Greer (1998). Color coding in the retina is also optimized to code quality as an intensity-invariant entity. In the retina, three types of sensory neurons create a space formed by two color-opponent channels, in which it is not the absolute activity, but rather the relative activity of the input channels, that is extracted. For example, in the human retina, there are two types of color-opponent ganglion cells that send their signals to the thalamus: either red-green or yellow-blue cells. Activity in these cells reflects the color properties of an object, to a large degree independent of its intensity. They are

calculated by the retina microcircuits as contrasts between sensory neurons. For example, the red-green channel receives antagonistic input from the middle-wavelength receptor ("green") and the long-wavelength receptor ("red"). Consequently, this channel is sometimes called "red-minus-green." The response properties of projection neurons and the innervation patterns of heterogeneous local interneurons are ideally suited for the necessary interactions among glomeruli. However, based on the multidimensional olfactory input space, we would expect a multidimensional odor-opponency output space with possibly quite complex response properties. What are the chances of there being a vanilla-minus-garlic-channel? This is an area where the lack in psychophysical knowledge is blatant. With the possible exception of some pheromone systems, there are as yet no documented examples of odor-opponency channels.

Odor Component Analysis

Most naturally occurring odors are complex blends of a large number of volatile compounds. Additionally, the turbulent nature of air leads to odor plumes being mixed in a chaotic fashion. This creates a conundrum for the olfactory system: synthetic representations of the blend or an analytical separation of the components. To investigate this issue, the responses to odor mixtures must be analyzed. Several studies have asked how many odors animals can differentiate in a mixture. However, the results cannot be generalized: Every perfumer knows that some odors blend, while others do not; thus the number of discriminable odors depends on the quality of the odors mixed. Electrophysiological studies have shown several kinds of mixture interactions. The presence of an odor B can interfere with the normally strong response to an odor A, which is termed mixture suppression. Conversely, a neuron or glomerulus may respond to a binary mixture with a response that exceeds the summed responses to the single components, defined as synergism. An inhibitory network within the antennal lobe geared at sharpening odor-response patterns should have the effect of creating stronger mixture interactions for mixtures of similar odors than for odors with completely different response patterns. Indeed, calcium imaging responses from honeybee projection neurons to binary mixtures showed inhibitory mixture interactions only for odors that are chemically closely related, such as alcohols which only slightly differed in carbon chain length. This effect was odor and glomerulus specific and could be modified pharmacologically, suggesting that heterogeneous local interneurons are the primary mediators of this effect (Sachse and Galizia, unpublished). Psychophysically, this means that similar odors in a mixture "compose" a new odor, which should make it difficult for the olfactory system to extract the single odor components (synthetic representation), while mixtures of dissimilar substances are represented as the sum of the optimized representation of each component (analytical representation). Indeed, behavioral data in rats support this idea by showing that rats perceive

binary mixtures composed of dissimilar odors as very similar to their components, whereas binary mixtures containing similar odors appeared dissimilar to the animal (Wiltrout et al. 2003). By increasing the number of components in an odor mixture, mixture interactions are likely to increase and further reduce the similarity to the single component patterns. With this coding strategy of odor mixtures, the olfactory system reveals a mechanism that allows representation of each possible odor mixture without saturating the olfactory code but losing analytical information.

Odor Concentration as a Continuous Variable

Animals can be trained to respond to an odor with a conditioned response (associative conditioning). If an animal is given a novel odor and responds to it, it has generalized from the trained odor to the novel one. Behavioral experiments show that animals generalize within the same odor over a wide range of concentrations (suggesting a concentration-invariant code) (Borst 1983) or only towards higher concentrations (Pelz et al. 1997). Other experiments have shown that bees can learn to distinguish tenfold concentrations from another, if sufficiently trained. This argues for the maintenance of the concentration information, albeit in a way that needs more training to be learned (Ditzen et al. 2003). Since the spatial activity patterns are widely concentration invariant, the intensity is unlikely to be coded in the spatial combination of activated cells/glomeruli. However, total activity intensity in the antennal lobe increases continuously with rising odor concentration (Sachse and Galizia 2003). In honeybees and flies, this information would be accessible for multiglomerular projection neurons. These neurons send their axons to the lateral protocerebrum (unlike the uniglomerular projection neurons, which innervate both the lateral protocerebrum and the mushroom bodies). Interestingly, at least parts of these neurons are GABAergic, which means that with increasing odor concentration they would inhibit increasingly their target neurons. Thus the coding of quality and intensity might already be separated by different neuron types in the honeybee antennal lobe and, later, even separated and processed in different brain structures.

Electrophysiological responses of projection neurons in locusts to different odor concentrations reveal a different situation (Stopfer et al. 2003). In these experiments, the response of a single projection neuron to different odor concentrations appeared unpredictable and did not correlate with odor intensity. Thus, each odor at each concentration had its own representation that seemed to be unrelated to that of the same odor at other concentrations. This is different from the continuous representation observed in honeybees. This difference might have morphological reasons: data in the honeybee were recorded from uniglomerular projection neurons, while locust projection neurons collect information from many glomeruli (Figure 13.2). Despite the striking difference in the responses to

odor concentrations in the two studies, the resulting representation of each odor in a multidimensional space either defined by the activated glomeruli (honeybee) or by the dynamics of different projection neurons (locust) is remarkably similar. In both cases the representation of odor quality is clearly separated into different clusters, whereas odor intensity appears in a continuous order within each quality cluster. Both studies agree on a separate representation of quality versus a continuous coding of intensity, even though the analysis of olfactory responses measured in the two species produced completely different response properties.

It is unknown how odor intensity is treated in the reformatting step between projection neurons and Kenyon cells. For example, in the responses to a series of different odor concentrations, the representation in the mushroom body lips may show some degree of concentration invariance. Our results from the antennal lobe allow for such a mechanism, since the relative activity of glomeruli is quite stable over several orders of magnitude (see above). Alternatively, as has been previously suggested (Heisenberg 2003), the increased overall activity found in the antennal lobe may lead to more Kenyon cells being activated.

Further Sharpening by Synchrony?

We have found that the antennal lobe optimizes odor representation, as described above (Sachse and Galizia 2002, 2003). A mathematical analysis of spatial activity patterns resulted in the best odor representation if only the strongest glomeruli were taken into account. This suggests that odor representation could even be improved if higher-order brain centers were to read out just the strongest projection neuron responses, raising the question of what mechanism could accomplish such a selective extraction of a subpattern. As mentioned earlier, coactivated projection neurons tend to synchronize in locusts due to the distributed action of GABAergic local interneurons. It is conceivable that projection neurons from strongly activated glomeruli may synchronize more than those from weakly activated ones. If the synchronous projection neuron spikes were the only information affecting higher-order brain centers, the olfactory system would have evolved a strategy for a selective readout. Nonsynchronous projection neuron spikes are probably not useless and are involved in processing mechanisms within the antennal lobe (see above) and/or in conveying information to brain areas other than the mushroom bodies, such as the lateral protocerebrum. An even simpler solution would exploit the statistics of projection neuron responses. Instantaneous firing frequencies of projection neurons go well into the hundreds of spikes/s. These neurons will always contribute with a spike in every readout epoch. Such a mechanism would create an efficient filter without the need of a dedicated network within the antennal lobe. Future experiments are necessary to elucidate the relationship of spatial and temporal features of olfactory responses.

PROPOSITIONS

Glomerular microcircuits are made up of a variety of morphologically distinct cells. We propose that processing is a property of the network. Attributing "tasks" to each cell type can only result in a caricature of the system's operation. Nevertheless, such simplifications aid an understanding of the basic mechanisms, and thus we reformulate the thoughts expressed above in terms of their most prominent cellular components.

1. *Gain setting: Homogeneous local interneurons*. Homogeneous local interneurons innervate the antennal lobe globally. They set the background activity close to the threshold, thus optimizing the system's sensitivity and preventing an overloading of the system.

2a. *Contrast enhancement: Heterogeneous local interneurons*. Heterogeneous local interneurons create contrasts between a single, densely innervated glomerulus (synaptic input) and a limited number of diffusely innervated glomeruli (synaptic output). They reduce the correlation between glomerular responses in a glomerulus-specific way, perhaps creating odor-opponency circuits.

2b. *Odor mixture interactions: Heterogeneous local interneurons*. One consequence is that similar odors blend in a way that makes extraction of component identity difficult (synthetic representation), while mixtures of dissimilar odors can still be analyzed (analytical representation).

3. *Continuous concentration readout: Multiglomerular projection neurons*. Multiglomerular projection neurons have access to the entire antennal lobe. They may respond to global activity, and thus give information about stimulus timing (onset/offset) and concentration.

4. *Selective readout: Uniglomerular projection neurons*. Uniglomerular projection neurons extract the activity in each glomerular microcircuit and project to higher-order brain centers. The identity of an odor is encoded in the combinatorial activity pattern across their identified axons.

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REFERENCES

Abel, R. 1997. Das olfaktorische System der Honigbiene: Elektrophysiologische und morphologische Charakterisierung von Antennallobusneuronen und deren Beteiligung beim olfaktorischen Lernen. Ph.D. Thesis. Berlin: Freie Universität Berlin.

Attwell, D., and S.B. Laughlin. 2001. An energy budget for signaling in the grey matter of the brain. *J. Cereb. Blood Flow Metab*. **21**:1133–1145.

Aungst, J.L., P.M. Heyward, A.C. Puche et al. 2003. Centre-surround inhibition among olfactory bulb glomeruli. *Nature* **426**:623–629.

Borst, A. 1983. Computation of olfactory signals in *Drosophila melanogaster. J. Comp. Physiol*. A **152**:373–383.

Brockmann, A., and B. Brückner. 1995. Projection pattern of poreplate sensory neurones in honeybee worker *Apis mellifera L.* Hymenoptera: Apidae. *Intl. J. Insect Morphol. Embryol.* **24**:405–411.

Christensen, T.A., B.R. Waldrop, and J.G. Hildebrand. 1998. Multitasking in the olfactory system: Context-dependent responses to odors reveal dual GABA-regulated coding mechanisms in single olfactory projection neurons. *J. Neurosci*. **18**:5999–6008.

de Bruyne, M., K. Foster, and J.R. Carlson. 2001. Odor coding in the *Drosophila* antenna. *Neuron* **30**:537–552.

Ditzen, M., J.F. Evers, and C.G. Galizia. 2003. Odor similarity does not influence the time needed for odor processing. *Chem. Senses* **28**:781–789.

Durbin, R., and G. Mitchison. 1990. A dimension reduction framework for understanding cortical maps. *Nature* **343**:644–647.

Fiala, A., T. Spall, S. Diegelmann et al. 2002. Genetically expressed cameleon in *Drosophila melanogaster* is used to visualize olfactory information in projection neurons. *Curr. Biol*. **12**:1877–1884.

Fonta, C., X.J. Sun, and C. Masson. 1993. Morphology and spatial distribution of bee antennal lobe interneurones responsive to odours. *Chem. Senses* **18**:101–119.

Friedrich, R.W., and G. Laurent. 2001. Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity. *Science* **291**:889–894.

Galizia, C.G., and B. Kimmerle. 2004. Physiological and morphological characterization of honeybee olfactory neurons combining electrophysiology, calcium imaging, and confocal microscopy. *J. Comp. Physiol. A.* **190**:21–38.

Galizia, C.G., A. Küttner, J. Joerges, and R. Menzel. 2000. Odour representation in honeybee olfactory glomeruli shows slow temporal dynamics: An optical recording study using a voltage-sensitive dye. *J. Insect Physiol*. **46**:877–886.

Galizia, C.G., and R. Menzel. 2001. The role of glomeruli in the neural representation of odours: Results from optical recording studies. *J. Insect Physiol*. **47**:115–130.

Hansson, B.S., and T.A. Christensen. 1999. Functional characteristics of the antennal lobe. In: Insect Olfaction, ed. B.S. Hansson, pp. 125–161. New York: Springer.

Heisenberg, M. 2003. Mushroom body memoir: From maps to models. *Nat. Rev. Neurosci*.**4**:266–275.

Hildebrand, J.G., and G.M. Shepherd. 1997. Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla. *Ann. Rev. Neurosci*. **20**:595–631.

Homberg, U., and U. Müller. 1999. Neuroactive substances in the antennal lobe. In: Insect Olfaction, ed. B.S. Hansson, pp. 181–206. New York: Springer.

Hudspeth, A.J., Y. Choe, A.D. Mehta, and P. Martin. 2000. Putting ion channels to work: Mechanoelectrical transduction, adaptation, and amplification by hair cells. *PNAS* **97**:11,765–11,772.

Laurent, G. 1999. A systems perspective on early olfactory coding. *Science* **286**: 723–728.

Laurent, G. 2002. Olfactory network dynamics and the coding of multidimensional signals. *Nat. Rev. Neurosci*. **3**:884–895.

Marin, E.C., G.S.X.E. Jefferis, T. Komiyama, H. Zhu, and L. Luo. 2002. Representation of the glomerular olfactory map in the *Drosophila* brain. *Cell* **109**:243–255.

Müller, D., R. Abel, R. Brandt, M. Zöckler, and R. Menzel. 2002. Differential parallel processing of olfactory information in the honeybee, *Apis mellifera L. J. Comp. Physiol. A* **188**:359–370.

Nässel, D.R. 1999. Histamine in the brain of insects: A review. *Microsc. Res. Tech*. **44**:121–136.

Ng, M., R.D. Roorda, S.Q. Lima et al. 2002. Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. *Neuron* **36**: 463–474.

Pelz, C., B. Gerber, and R. Menzel. 1997. Odorant intensity as a determinant for olfactory conditioning in honeybees: Roles in discrimination, overshadowing and memory consolidation. *J. Exp. Biol.* **200 (Pt 4)**:837–847.

Perez-Orive, J., O. Mazor, G.C. Turner et al. 2002. Oscillations and sparsening of odor representations in the mushroom body. *Science* **297**:359–365.

Sachse, S., and C.G. Galizia. 2002. Role of inhibition for temporal and spatial odor representation in olfactory output neurons: A calcium imaging study. *J. Neurophysiol.* **87**:1106–1117.

Sachse, S., and C.G. Galizia. 2003. The coding of odour-intensity in the honeybee antennal lobe: Local computation optimizes odour representation. *Eur. J. Neurosci*. **18**:2119–2132.

Shepherd, G.M., and C.A. Greer. 1998. Olfactory bulb. In: The Synaptic Organization of the Brain, ed. G.M. Shepherd, pp. 159–203. New York: Oxford Univ. Press.

Stopfer, M., S. Bhagavan, B.H. Smith, and G. Laurent. 1997. Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. *Nature* **390**:70–74.

Stopfer, M., V. Jayaraman, and G. Laurent. 2003. Intensity versus identity coding in an olfactory system. *Neuron* **39**:991–1004.

Stopfer, M., and G. Laurent. 1999. Short-term memory in olfactory network dynamics. *Nature* **402**:664–8.

Tanaka, N.K., T. Awasaki, T. Shimada, and K. Ito. 2004. Integration of chemosensory pathways in the *Drosophila* second-order olfactory centers. *Curr. Biol*. **14**:449–457.

Uchida, N., and Z.F. Mainen. 2003. Speed and accuracy of olfactory discrimination in the rat. *Nat. Neurosci.* **6**:1224–1229.

Vosshall, L.B. 2001. The molecular logic of olfaction in *Drosophila*. *Chem. Senses* **26**:207–213.

Wang, J.W., A.M. Wong, J. Flores, L.B. Vosshall, and R. Axel. 2003. Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. *Cell* **112**: 271–282.

Wilson, R.I., G.C. Turner, and G. Laurent. 2004. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science* **303**:366–370.

Wiltrout, C., S. Dogra, and C. Linster. 2003. Configurational and nonconfigurational interactions between odorants in binary mixtures. *Behav. Neurosci.* **117**:236–245.

Left to right: Charles Greer, Giovanni Galizia, Gilles Laurent, Pierre-Marie Lledo, Peter Mombaerts, Rainer Friedrich, and Silke Sachse (Stuart Firestein, not pictured)

14

Group Report: Olfactory Microcircuits

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OVERVIEW

Most organisms rely on an olfactory system to detect and analyze chemical cues in the environment in the context of essential behaviors. The basic layout of the first processing centers in the olfactory nervous system is remarkably similar in diverse phylogenetic classes, including insects and vertebrates. Chemicals are detected by odorant receptor proteins expressed by olfactory sensory neurons (OSNs), which send an axon to the first processing center in the brain, the olfactory bulb in vertebrates and the antennal lobe in insects. OSNs terminate in anatomically distinct input modules, the olfactory glomeruli. In all vertebrate and invertebrate species investigated to date, each OSN expresses only one or a few odorant receptors, and each glomerulus receives convergent input from only one type of OSN. Glomeruli are, therefore, considered functional units integrating sensory input from idiotypic afferents. Even simple odors stimulate multiple odorant receptors and thus evoke odor-specific patterns of afferent activity across the array of glomeruli. Within glomeruli, OSNs make excitatory synapses onto the output neurons, the mitral cells in vertebrates, and projection neurons in insects, as well as with local inhibitory interneurons. As a result of synaptic interactions within this network, the output of a given projection neuron is not simply determined by the sensory input to the glomeruli it innervates, but also by the activity of inputs channeled through other glomeruli. In addition, synaptic interactions temporally pattern olfactory bulb/antennal lobe output activity on at least two timescales. It is currently debated how odor information is encoded in the olfactory bulb/antennal lobe, and how neuronal circuits process odor information conveyed by sensory afferents. Moreover, the development and plasticity of olfactory circuits are only beginning to be elucidated. These issues are of particular interest because OSNs and interneurons in the olfactory bulb undergo continuous turnover throughout life in vertebrates.

FUNCTIONS AND CONSTRAINTS OF THE OLFACTORY SYSTEM

The function of any neuronal circuit can only be understood in the context of the operations it has to perform and by considering the constraints under which it operates. In other systems, it has proven fruitful to analyze their function under the assumption that sensory processing has evolved to detect statistical features of natural stimuli. For example, the receptive fields of visual or auditory neurons resemble basis functions optimized for the reconstruction of natural scenes or sounds, respectively (Olshausen and Field 1996; Lewicki 2002). The statistics of the olfactory stimulus space, however, have been analyzed only in a few specialized situations. Thus, correlations in the world of natural chemical stimuli are currently unknown. Some correlation in the response profiles of glomeruli is likely to result from the similarity of ligand binding by odorant receptors with overlapping tuning profiles. In a thought experiment, it is interesting to consider that the visual system recognizes an input pattern as a coherent object only when it has certain properties (e.g., contours delineating the shape of a house), but not when the input pixels are randomly distributed (e.g., "snow" on a TV screen). Similar considerations apply to the perception of sounds and noise by the auditory system. In olfaction, by contrast, it appears that any odorant or odorant mixture evokes a perception that is qualitatively similar to that evoked by a "meaningful" odor, similar to the perception of a mixture of colors as another color. The olfactory system may, therefore, not be specifically adapted to extract particular structure from a stimulus. Clearly, further insights into the statistics of odor stimuli and the interactions between odorant receptors and their ligands are required to understand the relationship between olfactory stimulus space and neural processing of odors.

Another consideration is that the operations performed by neuronal circuits should be reflected in the behavioral or psychophysical characteristics of the system. Humans cannot identify individual compounds in mixtures containing more than a few (3–4) components (Laing and Francis 1989). Rather, the perception of a mixture is either dominated by one intense component, or it acquires a novel character. Thus, the olfactory system appears to synthesize, rather than segment, information conveyed by different sensory channels. Sensory inputs through separate channels, such as glomeruli, therefore, likely interact during early processing in the brain.

CONSTITUENTS OF MICROCIRCUITS IN THE OLFACTORY BULB/ANTENNAL LOBE

The neuron types in the primary olfactory processing centers can be assigned to a relatively small number of classes in vertebrates and invertebrates (Table 14.1), although some differences occur across species and phyla (see Sachse and

Table 14.1 Brief overview of neuron types in the olfactory bulb/antennal lobe. In addition to sensory input from olfactory sensory neurons (OSNs), neurons in the olfactory bulb/antennal lobe also receive input from higher brain regions. These inputs are not reviewed here in any detail. For more comprehensive reviews of neurons and circuits in the olfactory bulb see Shipley and Ennis (1996), Shepherd and Greer (1998). Neurons in the invertebrate antennal lobe are reviewed in more detail by Sachse and Galizia (this volume). ACh: acetylcholine; GABA: gamma-aminobutyric acid; Glu: glutamate; M/T: mitral/tufted.

	Vertebrates			Insects		
	Known neuron classes	Transmitter phenotype	Properties	Known neuron classes	Transmitter phenotype	Properties
Sensory input	Olfactory sensory neurons	Excitatory (Glu, taurine)	Confined to glomerulus	Olfactory sensory neurons	Excitatory (ACh)	Confined to single glomerulus
Principal neurons	M/T cells	Excitatory (Glu)	Extensive extraglomerular dendrites; see text	Projection neurons	Excitatory	See text
Interneurons	Periglomerular cells ($\geq$ 5 subclasses)	Mostly inhibitory (GABA); some dopaminergic; some glutamatergic	Short or medium range	Local neurons ($\geq$ 5 classes)	Inhibitory (GABA, histamine)	Innervating all or subsets of glomeruli
	Granule cells ($\geq$ 2 subclasses)	GABAergic; small glutamatergic subpopulation	Small dendritic arbor; interacting with extensive M/T cell dendrites			
	Others: short axon cells, Van Gehuchten cells, unidentified cell	? Some glutamatergic				

Galizia, this volume). The principal neurons (mitral/tufted cells in vertebrates and projection neurons in invertebrates) receive sensory input and provide the output to higher brain regions. In many species (nonmammalian vertebrates and some invertebrates), a single principal neuron receives sensory input from a few glomeruli, whereas in mammals and other invertebrates (e.g., flies and bees), most adult principal neurons are uniglomerular. The vertebrate olfactory bulb further contains two classes of predominantly GABAergic interneurons, the periglomerular cells and the granule cells, each of which can be further subdivided. Morever, additional types of interneurons have been described in the olfactory bulb whose properties have not been examined extensively (Table 14.1). The antennal lobe of invertebrates contains GABAergic and, in some species, histaminergic interneurons that can be further subdivided into at least two classes based on their dendritic arborization in all or a subset of glomeruli.

MICROCIRCUITS IN THE OLFACTORY BULB/ANTENNAL LOBE

Microcircuits in the olfactory bulb/antennal lobe can be delineated at three levels:

1. Microcircuits on the synaptic scale are formed by reciprocal dendrodendritic synapses between principal neurons and inhibitory interneurons (Shepherd and Greer 1998). The principal neuron makes an excitatory synapse with an interneuron, which feeds back inhibition onto the principal neuron through an immediately adjacent synapse. In vertebrates, such microcircuits occur between mitral/tufted and periglomerular, as well as between mitral/tufted and granule cells. Another synaptic microcircuit consists of "synaptic triads" within glomeruli of vertebrates, where OSN input terminates on a periglomerular dendrite, which makes a synapse onto a mitral cell in the immediate vicinity. In insects, reciprocal dendrodendritic synapses and synaptic triads are formed by projection neurons and local neurons.
2. Glomeruli are viewed as microcircuits because they are among the most distinct anatomical modules in the brain and receive input from convergent, functionally uniform OSNs. Moreover, mitral cells associated with the same glomerulus are coupled by fast glutamate spillover and gap junctions at their apical dendritic tufts (Schoppa and Westbrook 2001; Urban and Sakmann 2002). In many species, glomeruli are encapsulated by a glial shell that may act as a diffusion barrier.
3. Microcircuits across glomeruli involve different interneurons. In insects, long-range interactions are mediated by inhibitory local neurons receiving synaptic input from OSNs and projection neurons and providing output to OSNs, local neurons, and projection neurons in some or all other glomeruli. In vertebrates, interactions beyond a single glomerulus are mediated by at least three different pathways. First, dendrites of periglomerular and short axon cells receive input from OSNs and mitral cells within a single glomerulus and provide dendritic output to the same, as well as axonal output to other glomeruli. Glutamatergic periglomerular and short axon cells appear to terminate on external tufted cells and presumably GABAergic periglomerular cells. These pathways extend over a short or medium spatial range and are presumed to mediate inhibition between sensory inputs to one glomerulus and mitral cells associated with the same and other glomeruli (Aungst et al. 2003). Second, mitral/tufted cells emit long-range axon collaterals that terminate on granule cells, which in turn contact distant mitral cells. Third, mitral cell synapses on extraglomerular dendrites stimulate granule cells, which in turn inhibit the same and other mitral cells. Because of the large extent of extraglomerular mitral/tufted dendrites, these interactions are long range.

The interglomerular circuits reviewed above all exert inhibitory effects on the principal neurons, which is different to other systems such as neocortex. Recent evidence from *Drosophila melanogaster*, however, suggests that lateral excitatory interactions between projection neurons also exist (Wilson et al. 2004), which would have important implications for circuit function. Currently, no candidate pathway mediating such interactions has been described in insects. In vertebrates, lateral excitation could be mediated by a small and transient glutamatergic subpopulation of granule cells (Didier et al. 2001) or by glutamate spillover between extraglomerular mitral cell dendrites (Isaacson 1999). Furthermore, the possible functions of the less intensively studied interneuron types (Table 14.1) remain to be elucidated.

PHYSIOLOGICAL FUNCTION OF MICROCIRCUITS

Reciprocal dendrodendritic microcircuits are assumed to mediate auto-inhibition of mitral cells, possibly without the need to elicit a spike in the interneuron (Chen et al. 2000; Lagier et al. 2004). Synaptic triads within glomeruli could effectively lead to a sign-inversion of OSN input onto mitral cells. Physiological evidence in moths indicates that two local neuron synapses can occur between OSNs and a projection neuron. Thus OSN input causes excitation of the projection neuron by disinhibition (Christensen et al. 1993).

The glomerular microcircuit is likely to perform multiple functions:

- The high convergence ratio of idiotypic OSNs onto principal neurons ($\sim$100:1 to 1000:1) is likely to increase the signal-to-noise ratio of the input channel and average out temporal noise.
- The coupling between apical dendrites is likely to distribute and amplify excitation across mitral cells, which may further increase the signal-to-noise ratio.
- Due to the glial barrier, the extracellular milieu within a glomerulus may be controlled independently of other glomeruli. During synaptic activity, the intraglomerular concentration of transmitters or potassium may change significantly (Jahr and Nicoll 1981). Furthermore, a change in the extracellular chloride concentration may alter the chloride reversal potential and change the effect of GABAergic synaptic transmission.
- GABA and dopamine released from periglomerular cells activate $GABA_B$ and D_2 receptors, respectively, on OSN nerve terminals in a paracrine fashion (Wachowiak and Cohen 1999; Aroniadou-Anderjaska et al. 2000; Ennis et al. 2001). This leads to a down-regulation of transmitter release and thus may mediate adaptation or gain control of individual input channels.
- Further physiological functions, for example, mediated by dendrodendritic interactions between periglomerular cells or local neurons and the principal neurons, may remain to be discovered (e.g., Hayar et al. 2004).

Multiple physiological functions have been associated with interglomerular circuits:

- Each principal neuron's spike output depends on the dendritic integration of sensory and interneuronal input. Interglomerular interactions are thus likely to shape the stimulus–response profile of output neurons in a complex fashion (see below).
- During an odor response, a fast and widespread oscillation is recorded in the local field potential that reflects the rhythmic synchronization of odor-specific subsets of neurons. This oscillatory activity is mediated by reciprocal interactions between principal neurons and inhibitory interneurons (MacLeod and Laurent 1996; Lagier et al. 2004). Inhibitory feedback onto principal neurons is provided by local neurons in insects and granule cells in vertebrates, is mediated by $GABA_A$ receptors, and does not appear to require sodium action potentials. Interglomerular interactions, therefore, underlie the synchronization of distributed, odor-specific sets of output neurons. In mammals, oscillatory synchronization appears to be facilitated by intrinsic resonant properties of mitral cells (Desmaisons et al. 1999).
- Distributed inhibitory feedback onto the output neurons may exert a function akin to gain control, both on the level of single output neurons' activity and on the level of the total output activity across the population (Friedrich and Laurent 2004).

Despite significant knowledge about microcircuits in the olfactory bulb/antennal lobe, their roles in the representation and processing of odor information are currently debated. The remainder of our report focuses on sensory representations and neural computations in the olfactory bulb/antennal lobe.

SPATIAL ORGANIZATION OF ODOR-EVOKED ACTIVITY

OSNs expressing the same odorant receptor converge onto one or a few glomeruli within the olfactory bulb/antennal lobe (Ressler et al. 1994; Vassar et al. 1994; Mombaerts et al. 1996) and appear to be functionally equivalent in their odor response properties (Wachowiak et al. 2004), thus establishing a spatial map of receptor expression. Multiple axon guidance mechanisms cooperate in this very precise targeting of OSN axons, including the odorant receptor itself (Mombaerts 2001). Recent results indicate that at least the final precision of glomerular targeting is controlled by homotypic interactions between odorant receptors expressed on OSN axon terminals (Mombaerts and Feinstein, this volume).

The number of glomeruli is correlated with the number of functional odorant receptor genes in different species. *Drosophila melanogaster* has at least 61

odorant receptor genes and 43 glomeruli, mice have ~1000 odorant receptor genes and ~2000 glomeruli, whereas rats have ~1500 functional odorant receptor genes and ~3000 glomeruli. In mammals, each odorant receptor is associated with, on average, ~2 glomeruli in each olfactory bulb. The roughly 1:1 correspondence between the number of odorant receptor genes and the number of functionally different glomeruli lead to the model that each glomerulus integrates the input to the olfactory bulb/antennal lobe conveyed by one receptor type. Glomeruli, therefore, represent separate input channels or dimensions that are, however, not orthogonal to each other.

In mammals, but not in lower vertebrates and invertebrates, the map of receptor expression is mirror symmetric about a roughly vertical plane; that is, most idiotypic OSNs project to glomeruli at similar coordinates in the medial and lateral hemisphere of each olfactory bulb. Moreover, external tufted cells receiving input from a given glomerulus project to locations in the granule cell layer in the vicinity of the homotypic glomerulus in the other olfactory bulb hemisphere. Currently, the functional importance, if any, of the mirror-symmetric organization of the mammalian bulb is unresolved.

The spatial coordinates of idiotypic glomeruli are preserved, but not with exquisite precision. Rather, the position of a given glomerulus can vary within 1–2% of the surface of the olfactory bulb across individuals and between hemispheres of the same olfactory bulb. As a consequence, immediate neighborhood relationships between glomeruli are variable (Strotmann et al. 2000).

Odor-evoked activity across the array of glomeruli has been visualized by a variety of techniques, including 2-deoxyglucose uptake, *c-fos* expression, fMRI, intrinsic signal imaging, and calcium imaging (Stewart et al. 1979; Guthrie et al. 1993; Friedrich and Korsching 1997; Johnson et al. 1999; Rubin and Katz 1999; Sachse et al. 1999; Meister and Bonhoeffer 2001; Wachowiak and Cohen 2001; Xu et al. 2003). Even single chemical compounds activate multiple glomeruli and single glomeruli respond to multiple odorants, presumably because each odorant receptor can be activated by multiple compounds (Araneda et al. 2000). Odor information is, therefore, contained in a combinatorial pattern of activity across the array of glomeruli. Patterns evoked by chemically related odorants are often similar. Thus, microcircuits in the olfactory bulb/antennal lobe must analyze spatially distributed activity patterns to extract stimulus information.

An important question is whether the spatial relationships between glomeruli in the map reflect similarities between the respective odorant receptors' response profiles. Such an organization could create a *chemotopic* map, in which features of the chemical stimulus space are spatially mapped onto the array of glomeruli. Glomeruli responding similarly to a subset of odors sharing obvious chemical features are sometimes clustered spatially. For example, in experiments using 2-deoxyglucose uptake, intrinsic signal imaging, and fMRI, aliphatic aldehydes were found to activate glomeruli in an anteromedial region of

the dorsal olfactory bulb. However, even within this region only a subset of glomeruli responded to aliphatic aldehydes, and these odors also stimulated glomeruli in other locations. Studies using imaging of calcium indicators or a transgenic fluorescent activity probe in OSN axon terminals (Wachowiak and Cohen 2001; Bozza et al. 2004) yielded only weak evidence for a chemotopy of aldehyde responses in the dorsal olfactory bulb. Moreover, individual glomeruli within a region loosely defined by its response to a class of odorants can also respond to other, dissimilar sets of stimuli. As a result, the spatial proximity of glomeruli appears to be only weakly correlated with the similarity of their overall response profile (Friedrich and Stopfer 2001). The structure of chemotopic maps is, therefore, not well understood and deserves further experimental attention. Nonetheless it is clear that a chemotopic organization, if it exists, is much more fractured than topographic maps in other sensory systems, possibly relating to the complexity and high dimensionality of the stimulus space (Friedrich and Stopfer 2001). It is also interesting to note that from the perspective of neuronal wiring, spatial distance between all glomeruli is equal in insects, because all interglomerular connections pass through the central area of the spherical antennal lobe (Sachse and Galizia, this volume).

It is now firmly believed that the identity of active units in the combinatorial pattern contains essential stimulus information. Currently unresolved, however, is the question whether the position per se of glomeruli in the map is important for the decoding of glomerular activity patterns. It is, for example, possible that the given arrangement of glomeruli simply minimizes the total wiring length of circuits in the olfactory bulb/antennal lobe or is a byproduct of the axon guidance mechanisms underlying glomerular targeting, that is, a developmental process. In a thought experiment, the shuffling of glomerular positions in the map does not affect the information conveyed by activity patterns. Moreover, the information could potentially be extracted in the same way after shuffling if all connections in the network were kept intact. However, the system may still require positional information for its function. For example, interactions through gap junctions or electrotonic mechanisms may require a particular spatial arrangement of functional units within the circuit. Furthermore, it is formally possible that the establishment of correct synaptic connections between target neurons and their inputs relies on axon guidance mechanisms that read positional cues and would be fooled when glomerular positions are scrambled. Currently, there is no conclusive evidence arguing either for or against a role for spatial position in olfactory system function. This is clearly one important open question. Ideally, the problem should be approached by (genetic) manipulation of glomerular positions without otherwise disturbing the system and subsequent tests of the olfactory system's performance. This is, however, beyond the current realms of experimental possibility.

In the deeper layers of the olfactory bulb, focal excitation of a few glomeruli produces a cone of activity that fans out with increasing depth (Guthrie et al.

1993). Hence, glomerular activation spreads laterally within the olfactory bulb and probably also in the antennal lobe (Wilson et al. 2004). It is still unresolved whether the olfactory bulb shares a columnar functional organization with other brain structures. A "reductionist" analysis of basic properties of olfactory microcircuits using focal stimuli may be fruitful to derive insights into the mechanisms by which microcircuits process more complex glomerular activity patterns evoked by realistic stimuli.

TEMPORAL PATTERNING OF OUTPUT FROM THE OLFACTORY BULB/ANTENNAL LOBE

Olfactory microcircuits are renowned for their temporal dynamics. Temporal patterning of the activity of output neurons on at least two timescales has been observed in all species studied, vertebrates and invertebrates alike: (a) slow, aperiodic modulations of firing rate on a timescale of one or a few hundred of milliseconds, and (b) fast oscillatory synchronization with a precision of a few milliseconds. Further temporal patterns are observed in some species.

Odor-evoked Slow Temporal Patterning

Output neurons respond to odor stimuli with modulations of their firing rate during the first hundreds of milliseconds after stimulus onset. These firing rate modulations can include successive excitatory and inhibitory epochs resulting from circuit interactions in the olfactory bulb/antennal lobe. In mammals, odor-specific modulations of firing probabilities occur during each breathing cycle. The mechanisms underlying slow temporal patterning of output neurons are still elusive. Candidate pathways mediating these effects include all of the interglomerular microcircuits mentioned above. In locusts, slow temporal patterns were not substantially affected by $GABA_A$ or $GABA_B$ antagonists (MacLeod and Laurent 1996).

The slow temporal modulation of firing probability evoked by one odor is different between output neurons, and different odors evoke distinct slow temporal firing patterns in the same output neuron. As a result, the pattern of activity (firing rate) across the population of output neurons evolves in an odor-specific manner after stimulus onset. After a rapid change during the initial phase of the odor response, activity patterns asymptotically approach a relatively stable state after a few hundred milliseconds.

In zebrafish, locusts, and possibly moths, it has been shown that the dynamic change of spiking activity patterns evoked by similar odors results in a decorrelation of activity patterns evoked by related odorants (Friedrich and Laurent 2001; Stopfer et al. 2003; Daly et al. 2004; Friedrich and Laurent 2004): immediately after response onset, activity patterns evoked by related odors are similar, possibly because output neurons are driven to a large extent by their sensory

inputs, which respond similarly to related stimuli. Subsequently, however, patterns of output activity change, following trajectories that are specific for each stimulus and diverge over time. As a result, activity patterns evoked by related stimuli become more distinct, and the discrimination of patterns becomes significantly more reliable during the first few hundred milliseconds of the response. Hence, olfactory microcircuits perform a computation (pattern decorrelation) that appears important for the discrimination of "odor images" across glomeruli.

Odor-evoked Fast Oscillatory Synchronization

Odor-evoked population activity in the olfactory bulb/antennal lobe has an oscillatory component with frequencies ranging between 15 and 40 Hz in insects and lower vertebrates, and in the beta (15–30 Hz) and gamma (30–100 Hz) range in mammals. This oscillatory synchronization is mediated by reciprocal interactions between principal neurons and inhibitory interneurons in interglomerular microcircuits (see above). The spatial pattern of this oscillatory activity is widespread and only weakly reflects the discrete pattern of glomerular input. Within each oscillation cycle, only an odor-specific subset of output neurons synchronizes, while others fire without any apparent temporal relation to the oscillation. Hence, odor-specific subsets of spikes transmitted to higher brain regions are synchronized.

Since the integration of synaptic inputs in neurons can be exquisitely sensitive to temporal proximity, synchronized spiking may transiently establish neuronal ensembles that carry particular information accessible by coincidence detection-based readout mechanisms. Indeed, Kenyon cells in the mushroom body receiving input from projection neurons in insects are efficient coincidence detectors (Laurent and Naraghi 1994; Perez-Orive et al. 2002). The short temporal integration window is established by two mechanisms. First, intrinsic mechanisms, probably involving voltage-gated Ca^{2+} and possibly Na^{+} channels, boost synaptic transients. Second, projection neurons also target a small pool of GABAergic neurons elsewhere in the brain, which in turn provides strong and nonspecific feedforward inhibition onto Kenyon cells. This inhibition arrives at the Kenyon cell dendrite with a delay relative to the excitatory projection neuron input during the same cycle, thereby defining a sharp integration time window. Each Kenyon cell receives input from a small fraction ($\sim$2.5 %) of the projection neuron population. Hence, Kenyon cells in the mushroom body analyze selectively synchronized spiking across an evolving subpopulation of neurons during each oscillation cycle (Laurent, this volume).

In vertebrates, little is known about the temporal integration properties of neurons downstream of the olfactory bulb. Moreover, while output from the antennal lobe is conveyed to only two target areas in insects, output from the olfactory bulb is transmitted to at least five different areas in vertebrates. Therefore, it is of prime importance to study the properties of neurons innervated by

mitral/tufted cells to understand which of the properties of the temporally structured pattern of activity across olfactory bulb outputs may be relevant for further processing.

The mechanisms of readout by Kenyon cells in the mushroom body suggest that these neurons selectively access information from synchronized spikes, while other spikes are discarded. This does not, however, imply that nonsynchronized spikes are irrelevant. They could, for example, play important roles in interglomerular microcircuits within the olfactory bulb/antennal lobe. Furthermore, it is possible that nonsynchronized spikes also convey information that may be retrieved by target neurons with longer integration time constants. Indeed, recent results support the hypothesis that patterns of nonsynchronized spikes convey important information accessible by using a longer integration window (Friedrich et al. 2004). These results suggest that due to the synchronization of subsets of output neurons, different messages may be multiplexed and conveyed simultaneously to higher brain regions by the across-neuron pattern of spiking in mitral/tufted cells.

The above considerations given to operations performed by olfactory microcircuits on afferent glomerular activity have important implications concerning the representation of stimulus information in the olfactory bulb/antennal lobe:

- In the inputs and the outputs of the olfactory bulb/antennal lobe, odor information conveyed by the response of single elements is limited because of their moderate odor selectivity. Rather, stimulus information has to be retrieved from the pattern of activity across many elements (glomeruli or output neurons). Microcircuits, therefore, appear to transform one combinatorial representation into another.
- Currently, it is uncertain whether the position of active units is necessary for the function of olfactory circuits. Theoretically, it is possible that the system relies on positional cues (e.g., during the development of connectivity), even though information is contained purely in the identity of active neurons. An experimental approach to this problem has thus far proven difficult.
- One computation appears to be a decorrelation of activity patterns by the dynamic distribution of activity across output neurons. The underlying synaptic mechanisms are, however, not known precisely.
- The transient synchronization of ensembles of output neurons is an important factor in determining the readout of antennal lobe activity by Kenyon cells in locusts. Synchronization may, therefore, play an important role in the transmission of information from the antennal lobe. Further results, however, are required to understand the role of oscillatory synchronization in other insect species and in vertebrates.
- Available evidence indicates that temporal activity patterns observed in output neurons reflect the dynamic reorganization of instantaneous

activity across the population. Theoretically, downstream neurons may also detect the temporal evolution of firing in single neurons or ensembles. However, there is currently no evidence for mechanisms supporting this hypothesis.

These considerations indicate that odor information resides in (a) the identity and instantaneous activity of elements in activity patterns and (b) their synchronization. These features would, therefore, be considered part of the "code." Other properties of odor-evoked activity in the olfactory bulb/antennal lobe, such as the position of glomeruli/neurons or the slow temporal patterning of activity, may not be analyzed directly by downstream targets; further results are needed to resolve this question. If so, they would constitute a "format" of odor-encoding activity patterns. Moreover, they are likely to play essential roles in important computations within the olfactory bulb/antennal lobe that affect the "code," such as the decorrelation of sensory inputs.

FUNCTION OF THE OLFACTORY BULB/ANTENNAL LOBE

Much contemporary research revolves around the general computations performed by microcircuits in the olfactory bulb/antennal lobe. It is, therefore, worth considering the function of the olfactory bulb/antennal lobe in more general terms. It is currently contended that in the periphery, odors are represented by distributed patterns of activity across OSNs or glomeruli. Due to overlapping response profiles of odorant receptors, and possibly due to correlations in the world of natural stimuli, these patterns are not evenly distributed within the neural space (in which each dimension represents the activity level of one OSN type/glomerulus). Patterns are instead clustered, complicating the discrimination of individual stimulus representations. This inherent structure in the world of peripheral odor representations leads to the assumption that one function of olfactory processing in the olfactory bulb/antennal lobe is to promote the separation of overlapping odor representations.

In the architecture of interglomerular microcircuits within the olfactory bulb, interactions resulting in inhibition of output neurons are prominent (see above). This fact gave rise to the hypothesis that lateral inhibition in the olfactory bulb/antennal lobe may contribute to odor discrimination. Drawing upon existing knowledge about other systems, the radial and horizontal processing of visual information in the retina has been suggested as a conceptual model. Horizontal cells modify the output of photoreceptors, whereas amacrine cells interact more directly with bipolar/ganglion cells (Shepherd and Greer 1998). In this analogy, the periglomerular cells are synonymous with horizontal cells and granule cells are equivalent to amacrine cells. This analogy leads to the hypothesis that the olfactory bulb/antennal lobe enhances contrast or detects edges in

odor-evoked patterns of sensory input by narrowing the response profiles of output neurons as compared to their inputs. Some experimental evidence supporting this hypothesis exists (Yokoi et al. 1995) but this evidence has recently been challenged (Laurent 1999). Moreover, recent analyses of the dynamics and reorganization of activity patterns are not consistent with a simple refinement of afferent "odor images." Despite some similarity in the general layout of circuits, the spatial retinal processing as a conceptual model for system function is, therefore, under debate. In another analogy to the retina, it has been proposed that odor processing in the olfactory bulb/antennal lobe may be more akin to the processing of colors by opponent channels. This proposal is intriguing and receives support from calcium imaging data (Sachse and Galizia, this volume). Due to the complexity of the olfactory stimulus space and the large number of channels (glomeruli), it is difficult to determine whether interglomerular microcircuits in the olfactory bulb establish "odor-opponency channels." Moreover, it does not account for the dynamic properties of olfactory bulb/antennal lobe output.

Another perspective has emerged recently, which views microcircuits in the olfactory bulb/antennal lobe as a (nonlinear) dynamical system (Laurent et al. 2001). The considered class of dynamical systems transforms stationary input patterns into time-varying output patterns, moving along input-specific trajectories in coding space (see Laurent, this volume). In this framework, a primary function of olfactory microcircuits would be to enable odor-specific dynamics that can decorrelate input patterns. Such a system would distribute clustered input patterns more evenly in coding space and, thus, optimize the use of the coding space for discrimination and other tasks. In this framework, the olfactory bulb/antennal lobe would reformat combinatorial representations so as to facilitate their readout. This view is generally consistent with the reorganization of odor-evoked olfactory bulb/antennal lobe output observed experimentally (Friedrich and Laurent 2001, 2004; Laurent 2002; Stopfer et al. 2003). Such a redistribution of activity in coding space would be considered successful (or "optimized") if single downstream neurons could immediately extract any relevant information from it. In other words, after evenly distributing representations in coding space, it should be possible to extract desired information by a simple classifier, such as a support vector machine (Fernandez Galan et al. 2004). Indeed, the extreme specificity of Kenyon cell odor responses in the locust indicates that very specific and high-level information can be extracted from the antennal lobe output in one synaptic step (Laurent, this volume).

An intriguing parallel is apparent between the dynamical systems view of the olfactory bulb/antennal lobe and liquid state machines, which have been proposed as a theoretical framework for the function of cortical circuits (Maass et al. 2002; Maas and Markram, this volume). While it is problematic to apply the liquid state machine model in its generalized form to the specific computations performed by the olfactory system, a more specialized form may lead to valuable theoretical insights into olfactory system function.

In summary, alternative general views of the function of olfactory microcircuits have emerged and are presently being debated vigorously. Common to these views is the notion that the olfactory bulb/antennal lobe does not extract highly specific information by creating outputs tuned very narrowly to particular stimuli or features. Rather, microcircuits appear to reformat odor representations for further use. Precisely how representations are reformatted, and what the use of the operations is for further processing, is controversial. According to one view, the output would be a refinement of afferent inputs without the need for dynamics, whereas under the other view, the output would be a fundamental reorganization of activity patterns requiring dynamics. Further research will certainly address these questions. Moreover, many views are motivated by the (perhaps subconscious) assumption that one primary goal of the olfactory system is to achieve fine odor discrimination, although olfactory circuits may, in addition, have evolved to achieve other tasks.

PLASTICITY OF OLFACTORY MICROCIRCUITS

Olfaction is often studied in the context of learning and memory. Interestingly, very few reports have appeared that describe synaptic plasticity associated with learning and memory in other systems, such as spike-timing-dependent synaptic modulation or structural dendritic plasticity in the olfactory bulb/antennal lobe. Nevertheless, experience-related plasticity is observed at multiple levels. For example, prenatal exposure of pregnant mothers to food odors causes enhanced sensory responses to these odors in pups after birth, and supervised and nonsupervised plasticity mechanisms can change odor-evoked spatial or temporal activity patterns in the olfactory bulb/antennal lobe (Freeman and Schneider 1982; Kendrick et al. 1992; Faber et al. 1999; Stopfer and Laurent 1999). The olfactory bulb/antennal lobe receives centrifugal inputs that express neuromodulators (acetylcholine, serotonin, and norepinephrine in vertebrates and octopamine, dopamine, and serotonin in insects). These neuromodulators act at different sites and may modulate the function of olfactory microcircuits in a concerted fashion (e.g., Castillo et al. 1999). Noradrenergic inputs have, in particular, been implicated in the local modulation of dendrodendritic synaptic microcircuits between mitral and granule cells in the context of olfactory memory formation (Kendrick et al. 1992). In insects, octopamine and dopamine are likely to be important neuromodulators in the antennal lobe and higher brain regions (Hammer and Menzel 1998; Schwaerzel et al. 2003).

A remarkable feature of the vertebrate olfactory system is the lifelong turnover of both OSNs and interneurons in the olfactory bulb (see Lledo, this volume). Neuronal turnover is not observed in the olfactory system of invertebrates, possibly because their lifespan is usually much shorter. The life span of a mature vertebrate OSN is about 90 days but can be prolonged to 12 months under certain conditions, indicating that it is regulated by environmental factors.

Blocking airflow through one naris reduces the formation of new neurons, raising the question as to which mechanisms control stem cell proliferation. One obvious role of ongoing turnover of OSNs is the replacement of OSNs that have been damaged by exposure to pathogens or otherwise. It is currently unknown whether the turnover of OSNs can also contribute to the adaptation of individuals to slow changes of the natural odor space.

Within the adult olfactory bulb, interneurons are continuously replaced by new neurons that originate from the subventricular zone and migrate to the olfactory bulb in the rostral migratory stream. In the embryo, bulbar interneurons are derived from neuronal precursors in a different proliferation zone, the lateral ganglionic eminences. Up to 80,000 new neurons arrive in the adult olfactory bulb every day, and ~1% of the granule cells are turning over at each moment. Conceivably, the turnover of neurons in the adult olfactory bulb could modify the function of olfactory microcircuits in important ways, on a timescale of weeks. Moreover, it is an interesting question how the function of olfactory microcircuits is maintained during the continuous integration of new neurons. New neurons in the olfactory bulb gradually mature over a period of ~4 weeks. However, under normal conditions, ~50% of the newborn neurons will undergo apoptosis within a few days following their integration in the network. The rate of apoptosis, but not the rate of turnover, depends on external factors (see below). Hence, the addition of new neurons to the olfactory bulb can be regulated by modulating neuron survival.

If newborn interneurons are necessary for bulbar function or plasticity, disruption of cell migration in the rostral migratory stream would be expected to affect olfactory processing or learning. Indeed, in PSA-NCAM-mutant mice, the number of newborn granule cells is reduced by ~40% and odor discrimination is impaired (Gheusi et al. 2000). One hypothesis is that the impairment is due to reduced GABAergic inhibition of mitral cells by granule cells, which is likely to play a role in the function of interglomerular microcircuits (see above). Furthermore, the rate of apoptosis is reduced in animals exposed to an enriched olfactory environment. This effect is associated with more robust and extended long-term memory measured in a simple task. In general, these results suggest a relationship between interneuron number and system performance.

The maturation of adult-generated neurons does not recapitulate the maturation of the same interneuron types during embryogenesis (Carleton et al. 2003). An important difference in the maturation of granule cells is that Na^+ channels conferring spiking activity are expressed early during maturation in the embryo but appear very late in the maturation of adult-generated granule cells. This may be a mechanism to prevent the interference of immature granule cells with the functional circuits already in place. An important step in the maturation of newborn neurons is the exit from the rostral migratory stream into the granule cell layer of the olfactory bulb. Because migration is predominantly radial after leaving the rostral migratory stream, this step determines the region where the new

neuron will be integrated. Exit from the rostral migratory stream is blocked by NMDA receptor antagonists, raising the possibility that the recruitment of newborn neurons is site-specific and regulated by activity in the olfactory bulb. Ongoing experiments, therefore, address the question of whether sensory experience or odor learning may recruit newborn neurons specifically to those microcircuits that participate in the relevant behavior.

CONCLUSIONS AND AVENUES FOR FUTURE RESEARCH

The function of the olfactory system has been studied at multiple levels, ranging from molecular, biophysical, and anatomical studies on individual neurons to systems function, theory, and behavior. Obviously, all of these levels are essential to arrive at an integrated understanding of the system with its various levels of microcircuit organization. In comparison to other neural systems, such as the cerebral cortex, some essential questions—especially at the systems level—appear easier to approach in the olfactory system. For example, the statistics of neural inputs to the system are directly measurable, and its output may be more directly interpretable in the context of behavior. It has, therefore, been proposed that the olfactory system provides an opportunity to obtain meaningful data at the systems level, which is often a bottleneck in our understanding of complex neural circuits. Such data would also provide a basis for detailed theoretical approaches.

Currently lacking is precise information on the connectivity matrices between individual neurons and neuron types, both within and beyond the olfactory bulb/antennal lobe. This is required to understand how system functions arise from the integration of neurons into circuits and should be addressed by anatomical and physiological studies.

Although the olfactory bulb/antennal lobe has been the main focus of this review, functional insights at other levels of the olfactory system are also of primary interest, both in their own right and for the interpretation of the function of the olfactory bulb/antennal lobe. To understand the goal of olfactory processing better, the statistics of the stimulus space and glomerular odor representations must be known. Addressing these issues entails the analysis of natural olfactory habitats and studying interactions between odorant receptors and their ligands. At the same time, knowledge about the mechanisms by which the output of the olfactory bulb/antennal lobe is read by higher brain structures is necessary to recognize relevant features of odor-encoding activity patterns. Especially in vertebrates, more experimental data are needed.

Although our report has emphasized the commonalities between olfactory circuit structure and function, obvious differences also occur between species. For example, in some species (most vertebrates and some insects), principal

neurons are multiglomerular, while in others, they are uniglomerular. By analyzing the correlation between structural and functional differences across species in a comparative approach, insights into the function of microcircuits or the contribution of their constituents may be derived.

The plasticity of microcircuits, particularly the ongoing replacement of interneurons throughout life in vertebrates, is a remarkable characteristic of the olfactory system. Further studies of this phenomenon are of interest both with respect to understanding neuronal plasticity and with respect to the function of stem cells and neurogenesis during adulthood.

Finally, many issues discussed above are currently under debate and need to be addressed further. For example, no general consensus has been reached with respect to the role of oscillatory synchronization, or about the fundamental computations performed in the olfactory bulb/antennal lobe. Furthermore, chemotopic maps and the possible role of position in olfactory system function deserve further attention. It is anticipated that multiple and extensive experimental and theoretical efforts will be needed to resolve these questions.

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REFERENCES

Araneda, R.C., A.D. Kini, and S. Firestein. 2000. The molecular receptive range of an odorant receptor. *Nat. Neurosci*. **3**:1248–1255.

Aroniadou-Anderjaska, V., F.M. Zhou, C.A. Priest, M. Ennis, and M.T. Shipley. 2000. Tonic and synaptically evoked presynaptic inhibition of sensory input to the rat olfactory bulb via GABA(B) heteroreceptors. *J. Neurophysiol.* **84**:1194–1203.

Aungst, J.L., P.M. Heyward, A.C. Puche et al. 2003. Centre-surround inhibition among olfactory bulb glomeruli. *Nature* **426**:623–629.

Bozza, T., J.P. McGann, P. Mombaerts, and M. Wachowiak. 2004. *In vivo* imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* **42**:9–21.

Carleton, A., L.T. Petreanu, R. Lansford, A. Alvarez-Buylla, and P.-M. Lledo. 2003. Becoming a new neuron in the adult olfactory bulb. *Nat. Neurosci*. **6**:507–518.

Castillo, P.E., A. Carleton, J.D. Vincent, and P.-M. Lledo. 1999. Multiple and opposing roles of cholinergic transmission in the main olfactory bulb. *J. Neurosci*. **19**: 9180–9191.

Chen, W.R., W. Xiong, and G.M. Shepherd. 2000. Analysis of relations between NMDA receptors and GABA release at olfactory bulb reciprocal synapses. *Neuron* **25**: 625–633.

Christensen, T.A., B.R. Waldrop, I.D. Harrow, and J.G. Hildebrand. 1993. Local interneurons and information processing in the olfactory glomeruli of the moth *Manduca sexta*. *J. Comp. Physiol. A* **173**:385–399.

Daly, K.C., G.A. Wright, and B.H. Smith. 2004. Molecular features of odorants systematically influence slow temporal responses across clusters of coordinated antennal lobe units in the moth Manduca sexta. *J. Neurophysiol*. **92**:236–254.

Desmaisons, D., J.-D. Vincent, and P.-M. Lledo. 1999. Control of action potential timing by intrinsic subthreshold oscillations in olfactory bulb output neurons. *J. Neurosci.* **19**:10,727–10,737.

Didier, A., A. Carleton, J.G. Bjaalie et al. 2001. A dendrodendritic reciprocal synapse provides a recurrent excitatory connection in the olfactory bulb. *PNAS* **98**:6441–6446.

Ennis, M., F.M. Zhou, K.J. Ciombor et al. 2001. Dopamine D2 receptor-mediated presynaptic inhibition of olfactory nerve terminals. *J. Neurophysiol*. **86**:2986–2997.

Faber, T., J. Joerges, and R. Menzel. 1999. Associative learning modifies neural representations of odors in the insect brain. *Nat. Neurosci*. **2**:74–78.

Fernandez Galan, R., S. Sachse, C.G. Galizia, and A.V. Herz. 2004. Odor-driven attractor dynamics in the antennal lobe allow for simple and rapid olfactory pattern classification. *Neural Comput*. **16**:999–1012.

Freeman, W.J., and W. Schneider. 1982. Changes in spatial patterns of rabbit olfactory EEG with conditioning to odors. *Psychophysiology* **19**:44–56.

Friedrich, R.W., C.J. Habermann, and G. Laurent. 2004. Multiplexing using synchrony in the zebrafish olfactory bulb. *Nat. Neurosci*. **8**:862–871.

Friedrich, R.W., and S.I. Korsching. 1997. Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. *Neuron* **18**: 737–752.

Friedrich, R.W., and G. Laurent. 2001. Dynamic optimization of odor representations in the olfactory bulb by slow temporal patterning of mitral cell activity. *Science* **291**:889–894.

Friedrich, R.W., and G. Laurent. 2004. Dynamics of olfactory bulb input and output activity during odor stimulation in zebrafish. *J. Neurophysiol*. **91**:2658–2669

Friedrich, R.W., and M. Stopfer. 2001. Recent dynamics in olfactory population coding. *Curr. Opin. Neurobiol.* **11**:468–474.

Gheusi, G., H. Cremer, H. McLean et al. 2000. Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. *PNAS* **97**:1823–1828.

Guthrie, K.M., A.J. Anderson, M. Leon, and C. Gall. 1993. Odor-induced increases in c-fos mRNA expression reveal an anatomical "unit" for odor processing in olfactory bulb. *PNAS* **90**:3329–3333.

Hammer, M., and R. Menzel. 1998. Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learn. Mem*. **5**:146–156.

Hayar, A., S. Karnup, M.T. Shipley, and M. Ennis. 2004. Olfactory bulb glomeruli: External tufted cells intrinsically burst at theta frequency and are entrained by patterned olfactory input. *J. Neurosci*. **24**:1190–1199.

Isaacson, J.S. 1999. Glutamate spillover mediates excitatory transmission in the rat olfactory bulb. *Neuron* **23**:377–384.

Jahr, C.E., and R.A. Nicoll. 1981. Primary afferent depolarization in the in vitro frog olfactory bulb. *J. Physiol.* **318**:375–384.

Johnson, B.A., C.C. Woo, E.E. Hingco, K.L. Pham, and M. Leon. 1999. Multidimensional chemotopic responses to n-aliphatic acid odorants in the rat olfactory bulb. *J. Comp. Neurol.* **409**:529–548.

Kendrick, K.M., F. Levy, and E.B. Keverne. 1992. Changes in the sensory processing of olfactory signals induced by birth in sleep. *Science* **256**:833–836.

Lagier, S., A. Carleton, and P.-M. Lledo. 2004. Interplay between local GABAergic interneurons and relay neurons generates gamma oscillations in the rat olfactory bulb. *J. Neurosci*. **24**:4382–4392.

Laing, D.G., and G.W. Francis. 1989. The capacity of humans to identify odors in mixtures. *Physiol. Behav.* **46**:809–814.

Laurent, G. 1999. A systems perspective on early olfactory coding. *Science* **286**: 723–728.

Laurent, G. 2002. Olfactory network dynamics and the coding of multidimensional signals. *Nat. Rev. Neurosci.* **3**:884–895.

Laurent, G., and M. Naraghi. 1994. Odorant-induced oscillations in the mushroom bodies of the locust. *J. Neurosci*. **14**:2993–3004.

Laurent, G., M. Stopfer, R.W. Friedrich et al. 2001. Odor coding as an active, dynamical process: Experiments, computation and theory. *Ann. Rev. Neurosci.* **24**:263–297.

Lewicki, M.S. 2002. Efficient coding of natural sounds. *Nat. Neurosci*. **5**:356–363.

Maass, W., T. Natschlager, and H. Markram. 2002. Real-time computing without stable states: A new framework for neural computation based on perturbations. *Neural Comput*. **14**:2531–2560.

MacLeod, K., and G. Laurent. 1996. Distinct mechanisms for synchronization and temporal patterning of odor-encoding neural assemblies. *Science* **274**:976–979.

Meister, M., and T. Bonhoeffer. 2001. Tuning and topography in an odor map of the rat olfactory bulb. *J. Neurosci.* **21**:1351–1360.

Mombaerts, P. 2001. How smell develops. *Nat. Neurosci.* **4**:1192–1198.

Mombaerts, P., F. Wang, C. Dulac et al. 1996. Visualizing an olfactory sensory map. *Cell* **87**:675–686.

Olshausen, B.A., and D.J. Field. 1996. Emergence of simple-cell receptive field properties by learning a sparse code for natural images. *Nature* **381**:607–609.

Perez-Orive, J., O. Mazor, G.C. Turner et al. 2002. Oscillations and sparsening of odor representations in the mushroom body. *Science* **297**:359–365.

Ressler, K. J., S.L. Sullivan, and L.B. Buck. 1994. Information coding in the olfactory system: Evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* **79**:1245–1255.

Rubin, B.D., and L.C. Katz. 1999. Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* **23**:499–511.

Sachse, S., A. Rappert, and C.G. Galizia. 1999. The spatial representation of chemical structures in the antennal lobe of honeybees: Steps towards the olfactory code. *Eur. J. Neurosci.* **11**:3970–3982.

Schoppa, N.E., and G.L. Westbrook. 2001. Glomerulus-specific synchronization of mitral cells in the olfactory bulb. *Neuron* **31**:639–651.

Schwaerzel, M., M. Monastirioti, H. Scholz et al. 2003. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *J. Neurosci*. **23**:10,495–10,502.

Shepherd, G.M., and C.A. Greer. 1998. The olfactory bulb. In: The Synaptic Organization of the Brain, ed. G.M. Shepherd, pp. 159–203. New York: Oxford Univ. Press.

Shipley, M.T., and M. Ennis. 1996. Functional organization of olfactory system. *J. Neurobiol.* **30**:123–176.

Stewart, W.B., J.S. Kauer, and G.M. Shepherd. 1979. Functional organization of rat olfactory bulb analysed by the 2-deoxyglucose method. *J. Comp. Neurol.* **185**:715–734.

Stopfer, M., V. Jayaraman, and G. Laurent. 2003. Intensity versus identity coding in an olfactory system. *Neuron* **39**:991–1004.

Stopfer, M., and G. Laurent. 1999. Short-term memory in olfactory network dynamics. *Nature* **402**:664–668.

Strotmann, J., S. Conzelmann, A. Beck et al. 2000. Local permutations in the glomerular array of the mouse olfactory bulb. *J. Neurosci.* **20**:6927–6938.

Urban, N.N., and B. Sakmann. 2002. Reciprocal intraglomerular excitation and intra- and interglomerular lateral inhibition between mouse olfactory bulb mitral cells. *J. Physiol.* **542**:355–367.

Vassar, R., S.K. Chao, R. Sitcheran et al. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* **79**:981–991.

Wachowiak, M., and L.B. Cohen. 1999. Presynaptic inhibition of primary olfactory afferents mediated by different mechanisms in lobster and turtle. *J. Neurosci.* **19**:8808–8817.

Wachowiak, M., and L.B. Cohen. 2001. Representation of odorants by receptor neuron input to the mouse olfactory bulb. *Neuron* **32**:723–735.

Wachowiak, M., W. Denk, and R.W. Friedrich. 2004. Functional organization of sensory input to the olfactory bulb glomerulus analyzed by two-photon calcium imaging. *PNAS* **101**:9097–9102.

Wilson, R.I., G.C. Turner, and G. Laurent. 2004. Transformation of olfactory representations in the Drosophila antennal lobe. *Science* **303**:366–370.

Xu, F., N. Liu, I. Kida et al. 2003. Odor maps of aldehydes and esters revealed by functional MRI in the glomerular layer of the mouse olfactory bulb. *PNAS* **100**:11,029–11,034.

Yokoi, M., K. Mori, and S. Nakanishi. 1995. Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb. *PNAS* **92**:3371–3375.

15

Anatomical and Molecular Heterogeneity of Cortical GABAergic Interneurons

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ABSTRACT

It is generally considered that the basic cortical microcircuit is composed of a pyramidal cell and its input-output connections. The inhibitory inputs in these circuits mostly originate from GABAergic interneurons, whose terminations contact with the dendrites, soma, and initial axon segment of pyramidal cells. There is a great diversity of morphological types of interneurons, based on the morphology of their somata, dendritic, and axonal arborization patterns. Interneurons do not necessarily form synapses exclusively with other particular types of neurons, nor are their synapses restricted to pyramidal or non-pyramidal cells, or a single postsynaptic region. However, they do generally show a preference for certain postsynaptic partners. Most interneurons contain GABA, as well as a variety of other neurotransmitters, neuroactive peptides, and calcium-binding proteins. Indeed, one of the main conclusions from the collective work of a number of laboratories is that different types of interneurons are characterized by a particular combination of molecular, physiological, and synaptic features. These features enable the different interneuron subtypes to contribute to the generation of synchronized network activity thereby shaping principal cell behavior. Finally, it has become apparent that not all types of interneurons are found in all species and that molecular characteristics of interneurons may differ between species. Therefore, caution should be taken when extrapolating data on interneuronal circuitry from one species to another.

INTRODUCTION

Through the use of the "*reazione nera*" (black reaction), the method discovered by Camillo Golgi (1843–1926) in 1873, he became the first histologist to propose the existence of two morphologically and physiologically different types

of neurons in the nervous system: motor (type I) and sensory (type II) neurons. Type I neurons had long axons that left the gray matter (projection neurons), whereas type II neurons had short axons whose arbors remained near the parent cell and did not leave the gray matter (intrinsic neurons). Santiago Ramón y Cajal (1852–1934) argued that a number of situations arose in the nervous system that made this physiological distinction inaccurate. Furthermore, the axon of the type I motor neurons gave rise to axonal collaterals (local plexus) in their trajectory out of the site where the parent neurons were localized. That is, the axon of the motor neurons differed morphologically from those of the sensory type in its length. Thus, Cajal named Golgi's two types as cells with a *long axon* and cells with a *short axon*. Since then, the term short-axon cell has commonly been considered as synonymous with the term interneuron (DeFelipe 2002). However, because the axon of all neurons gives rise to local axonal arborization, referring to interneurons as "local circuit neurons" is incorrect.

The collective work of numerous laboratories has shown that in the cerebral cortex, two main classes of interneurons can be distinguished. These are commonly referred to as: spiny non-pyramidal or stellate cells and aspiny or sparsely spiny non-pyramidal cells. Spiny stellate cells are morphologically heterogeneous and are typically found in the middle cortical layers (especially layer IV). The axons of these cells branch within layer IV where their parent cell bodies are located, or in the layers above or below. These neurons form asymmetrical synapses with dendritic spines and shafts, and are considered to be excitatory. Aspiny or sparsely spiny non-pyramidal cells are highly varied in terms of their morphology, and they are found in all cortical layers. The axons of these neurons maintain their arbors near the parent cell, but they may additionally give rise to collaterals that course along a horizontal and/or vertical trajectory (descending and/or ascending). These aspiny or sparsely spiny non-pyramidal cells form symmetrical synapses with a variety of postsynaptic elements, but some specific morphological types can be grouped together due to their unique patterns of connectivity. These non-pyramidal cells constitute the majority of short-axon cells and approximately 15–30% of the total neuron population. Most of them appear to be GABAergic and thus inhibitory. In this chapter, we address certain anatomical, biochemical, and physiological characteristics of aspiny interneurons in the neocortex, which for simplicity we will refer to as interneurons.

ANATOMICAL DIVERSITY OF INTERNEURONS

Based on the morphology of their somata, and of their dendritic and axonal arborizations, there is great diversity of interneurons. The problem of classifying cortical interneurons has long been a subject for discussion (DeFelipe 2002), and a satisfactory consensus in classifying these neurons remains to be reached. In particular, there is no established convention for assessing which morphological characteristics of a neuron are essential to pertain to a given cell type or, in

other words, what morphological differences are functionally important. For instance, there are interneurons that display the same somatodendritic morphology but that have different patterns of axonal arborization. Conversely interneurons with the same axonal patterns but with different somatodendritic morphologies also exist (e.g., Jones 1975; Fairén et al. 1984). Nevertheless, certain interneurons can be recognized by their unique morphological characteristics, or they can be included into subgroups on the basis of their patterns of axonal arborization or the synaptic connections they establish with other interneurons and/or with pyramidal cells (Cajal 1904; Lorente de Nó 1922; Jones 1975; Fairén et al. 1984; DeFelipe and Jones 1988; White 1989; Lund 1990; Somogyi et al. 1998; Kawaguchi and Kondo 2002; Thomson and Deuchars 1997; Támas et al. 1997; Gupta et al. 2000; Silberberg et al. 2002). Furthermore, interneurons are not only connected by point-to-point chemical synapses, but some subpopulations may also be coupled electrically through gap junctions (e.g., Galarreta and Hestrin 1999; Gibson et al. 1999; Tamás et al. 2000; Amitai et al. 2002; Blatow et al. 2003; Fukuda and Kosaka 2003). Thus, the type of chemical and electrical connectivity should be included in the schemes of cortical circuits.

Morphological Characteristics of Interneurons

The first clear morphological attribute that can be used to distinguish an interneuron is the non-pyramidal cell morphology of the soma and dendritic arborization. There are three main morphological types of somata: ovoid, fusiform, and triangular. Similarly, three major dendritic morphologies can be recognized (Figure 15.1a):

- Multipolar: neurons with several dendrites radiating in all directions.
- Bitufted: neurons with two main dendrites running in opposite directions, which, after a relatively short trajectory, resolve into two dendritic tufts.
- Bipolar: cells with two principal long dendrites running in opposite directions and showing few dendritic collaterals.

With the exception of chandelier cells, which show a characteristic specialization of their terminal axonal branches giving rise to candlesticks or chandelier terminals (short, vertical rows of boutons), interneurons do not show any distinctive specialization of their axon terminals. This is even true for basket cells or neurons that contribute to basket formations, which involve the formation of a plexus of axons that typically surround the cell bodies of pyramidal cells. These formations originate from the convergence of axons of several cells, but the individual axon of a given cell contributing to the basket formation has no distinguishing morphology such that it can be identified as a basket terminal (Marin-Padilla 1969; Jones 1975; Fairén et al. 1984). Indeed, it is for this reason that basket cells are difficult to identify.

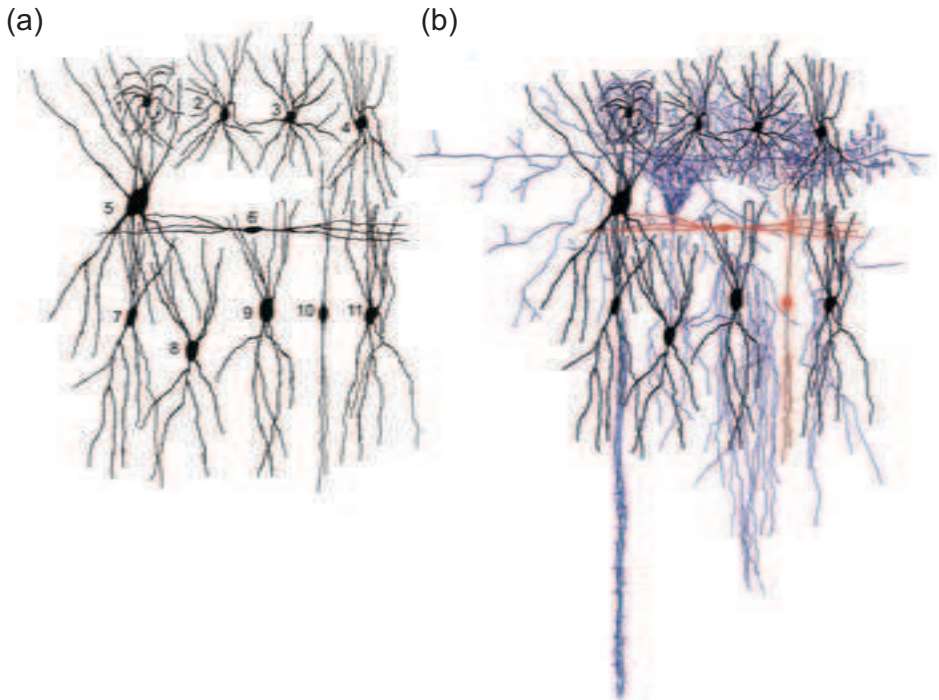

Figure 15.1 Drawings of the main types of cortical interneurons. (a) Only the somata and dendritic arborization are presented in black. (b) The axonal arborization is included (blue) except for the horizontal type I and II (6) and bipolar (10) neurons, whose pattern of axonal arborization is unknown. The latter two neurons are shown in red. 1, neurogliaform cells; 2, small basket cells; 3, common type I; 4, chandelier cells; 5, large basket cells; 6, horizontal bitufted neurons; 7, double bouquet cells; 8, Martinotti cells; 9, neurons with axonal arcades; 10, bipolar cells; 11, common type II.

The main morphological types of interneurons, which can be described using the Golgi method or by using intracellular labeling of individual neurons, can be included in three major groups (reviewed in Fairén et al. 1984; White 1989; DeFelipe and Jones 1988; Lund 1990; Kisvárday 1992; Kawaguchi and Kondo 2002; Somogyi et al. 1998; Thomson and Deuchars 1997; DeFelipe 2002; Silberberg et al. 2002). These groups are established on the basis of their general pattern of axonal arborization (in most cases) or by their unique somatodendritic morphology (Figure 15.1):

Group 1: Neurons with a restricted local axonal arborization, predominantly within the dendritic field.

- Neurogliaform cells: Multipolar small or medium size neurons in layers II–VI, characterized by their very local and highly branched dendritic and axonal arborization.
- Small basket cells: Multipolar or bitufted cells in layers II–VI, distinguished by the presence of numerous curved preterminal axonal branches.
- Common type 1: Multipolar or bitufted cells in layers II–VI that are small or medium sized and whose axons give rise to relatively short horizontal, oblique, and vertical collateral branches without any apparent preference.
- Chandelier cells: Multipolar or bitufted cells in layers II–VI, distinguished by having preterminal axonal branches that form short vertical rows of boutons resembling candlesticks.

Group 2: Neurons with prominent long horizontal axon collaterals and/or dendrites.

- Large basket cells: Multipolar or bitufted cells found mostly in layers III–V, and characterized by their large somata, long dendrites, and lengthy, horizontally oriented, myelinated axon collaterals that can reach a length of several hundred microns.
- Horizontal type I neurons: Horizontally bitufted neurons in layer I whose axons have not been characterized.
- Horizontal type II neurons: Horizontally oriented bitufted neurons in layers II–VI whose axons have not been characterized.

Group 3: Neurons whose axons give rise to conspicuous vertical collaterals and neurons with long, vertically oriented dendrites.

- Double bouquet cells: Multipolar or bitufted cells typically found in layers II–III and distinguished by their axons that form tightly intertwined bundles of long descending vertical collaterals.
- Martinotti cells: Multipolar or bitufted cells mostly found in layers V–VI. They are distinguished by their ascending axons which give rise to two plexus: one near the cell body and the other at a variable distance above the

cell body. This second plexus may be very dense (axonal tuft) or more diffuse, and can be found either in the same cortical layer as the cell body of origin, or more widely distributed in the layers above (ascending axons can travel from layer VI to layer I).

- Neurons with axonal arcades: Multipolar or bitufted cells found mostly in layers II–IV whose axons give rise to axonal arcades, producing predominantly vertical axonal arborizations and relatively long descending collaterals.
- Bipolar cells: Cells mostly found in layers II–IV characterized by a bipolar dendritic arborization, which can expand across several layers in the vertical plane. Their axons have not been characterized.
- Common type II: Multipolar or bitufted cells mostly found in layers II–IV characterized by their long vertically oriented dendritic fields, and by axons that give rise to a loose plexus of descending and/or ascending collaterals without any apparent preference.

INTERNEURONS' CONNECTIONS

Two aspects of the connectivity of interneurons can be distinguished with respect to important functional implications:

Level 1: Regional and spatial dendrite selectivity, including the region of the neuron where synapses form (i.e., dendritic shafts, dendritic spines, soma, or axon initial segment). At this level, the distance of the innervated dendritic segment from the cell body should be taken into account (i.e., proximal, intermediate, and distal portions), as should the order of the dendrite branch (i.e., primary, secondary, tertiary, etc.).

Level 2: Selective neuronal connectivity; that is the type of pyramidal or interneuron to which the cell is connected, either through chemical and/or electrical synapses. These should be identified by their morphology, localization (layer), molecular characteristics, synaptic connections, and in the case of pyramidal cells, by their projection sites.

With the exception of chandelier cells, which establish synapses exclusively with the axon initial segment of pyramidal cells, the other types of interneurons so far examined form synapses with both pyramidal and non-pyramidal cells (Figure 15.2). Even the same basket terminals have been shown to form synapses with the somata of a pyramidal neuron and with that of an adjacent GABAergic interneuron (DeFelipe et al. 1986). In addition, apart from chandelier cells, all interneurons have been found to establish synapses with more than one postsynaptic region (dendritic shafts, spines, somata, or axon initial segments). Furthermore, certain interneurons have been shown to form functional and strong autapses (synapses made by a neuron onto itself), which indicates that these interneurons are involved in the synaptic regulation of their own

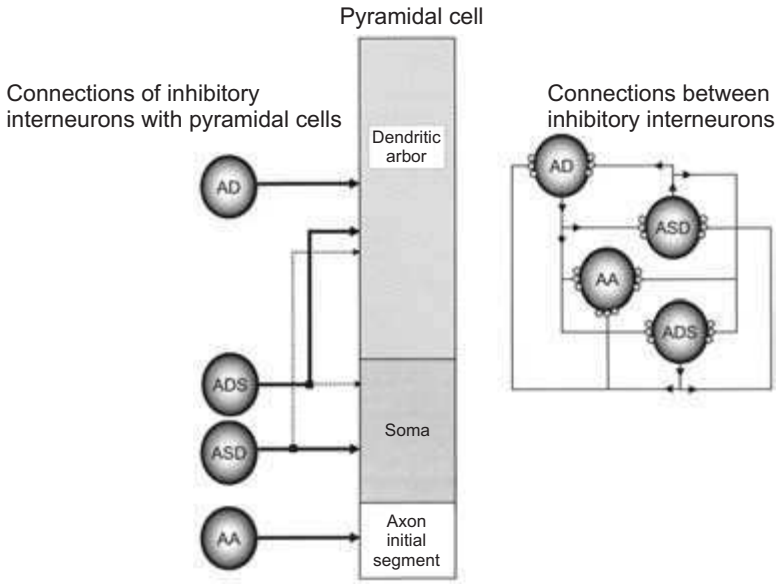

Figure 15.2 The general synaptic relationships between interneurons and pyramidal cells are depicted on the left. Four different groups of interneurons can be recognized: *axo-dendritic cells* (AD) or cells forming synapses only (or almost only) with dendrites (shafts and spines); *axo-somato-dendritic cells* (ASD) or cells forming multiple synapses with both dendrites and the somata, but with a preference for somata; *axo-dendrosomatic cells* (ADS) or cells forming multiple synapses with both dendrites and the somata, but with a preference for dendrites; and *axo-axonic cells* (AA) or cells forming synapses only with the axon initial segments (chandelier cells). Connections between interneurons are depicted on the right. All interneurons are presumably connected with other interneurons, except AA cells (chandelier cells), which only connect with pyramidal neurons.

activity as well as that of other neurons, through a powerful mechanism of self-innervation (e.g., Tamás et al. 1997; Bacci et al. 2003a). Thus, as a general rule, interneurons do not necessarily form synapses exclusively with other neurons. Neither do they show specificity for any single type of postsynaptic cell, pyramidal or non-pyramidal, nor for any single postsynaptic region. Rather, it appears that interneurons show preferences for certain postsynaptic partners.

On the basis of the preferred postsynaptic region of the pyramidal neuron, four different groups of interneurons can be recognized (DeFelipe 2002):

- *Axo-dendritic cells* (AD cells) or cells forming synapses only (or almost only) with dendrites (shafts and spines);
- *Axo-somatodendritic cells* (ASD cells) or cells forming multiple synapses with both the dendrites and somata, but with a preference for somata;
- *Axo-dendrosomatic cells* (ADS cells) or cells forming multiple synapses with both the dendrites and somata, but with a preference for dendrites;

- *Axo-axonic cells* (AA cells) or cells forming synapses only with the initial axon segment (chandelier cells; Figure 15.2).

In most cases, the postsynaptic cells are either poorly characterized at level 1, or not known at all, and very little information is available at level 2; that is, most details of interneuron circuitry remain unknown.

BIOCHEMICAL DIVERSITY OF INTERNEURONS

In general, it is thought that most interneurons contain GABA; however, an unknown proportion of interneurons do not express this neurotransmitter (e.g., Trottier et al. 1989; Del Río and DeFelipe 1997). In addition, other neurotransmitters (or their synthesizing enzymes) and a number of neuroactive peptides have been found in interneurons, the most frequent being: nitric oxide synthase (NOS), tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), somatostatin (SOM), cholecystokinin (CCK), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), and tachykinins (TK). Therefore, one of the major aims in the study of cortical circuitry is to try to characterize the different morphological types of interneurons at the molecular level, a fundamental step in understanding their function in the processing of information in the cortex.

The application of double-labeling immunohistochemistry in the 1980s revealed that a given neuron may express one or several transmitters and/or neuroactive substances (for a review, see Jones and Hendry 1986). Indeed, it became clear that interneurons are biochemically heterogeneous, as illustrated by the degree of co-expression of the most common neurotransmitters, neuropeptides, and the calcium-binding proteins calbindin (CB), parvalbumin (PV), and calretinin (CR; see Table 15.1). Together with the anatomical and physiological diversity of interneurons, this feature represents a major barrier when studying their role in cortical circuits. Thanks to the introduction of new technologies, a number of laboratories have been able to determine the functional implications of the different morphological, chemical, and physiological characteristics of certain interneurons. Using different methods for intracellular recording, the subsequent labeling of individual neurons, and in combination with other techniques, such as immunocytochemistry, *in situ* hybridization, single-cell reverse transcriptase polymerase chain reaction (RT-PCR), and electron microscopy (Monyer and Markram 2004), great advances have been made in our understanding of the physiological, molecular, and synaptic organization of interneurons (see below).

Biochemical Characterization of Morphologically Identified Interneurons

The soma, proximal dendrites, and axon initial segment of pyramidal cells are outlined by GABA and glutamatergic axon terminals (Alonso-Nanclares et al. 2004). However, all axon terminals forming synapses with the perisomatic

Table 15.1 Quantitative studies of co-localization of transmitters (or their synthesizing enzymes), neuropeptides, and calcium-binding proteins. Numbers in brackets refer to references listed on pages 306 and 308.

	GABA/ GAD	**ChAT**	**TH**
GABA/ GAD			
ChAT	*Rat*: 53% (30); 85% (3)		
TH	*Human*: 50% (48)		
CCK	*Monkey*: 90–95% (22) *Cat*: 89% (13); 100% (22, 44)		
NPY	*Monkey*: 90–95% (22) *Cat*: 100% (13, 22) *Rat*: 100% (33)		
VIP	*Monkey*: >90% (18) *Cat*: 0% (13) *Rat*: 100% (3, 33)	*Rat*: 19% (7); 34% (3); 47% (46)	
SOM	*Monkey*: 90–95% (22) *Cat*: 89% (13); 100% (22, 44) *Rat*: 80–85% (36); 90% (19); 100% (33) *Mouse*: 94% (47)		
nNOS	*Rat*: 88% (19); 100% (33)		*Human*: 27% layers V–VI (4)
PV	*Monkey*: 75–100% (5); 96% (24); 100% (49) *Cat*: 93% (15); 100% (25) *Rat*: 100% (19, 26, 27, 33, 39, 45)		
CB	*Human*: 71% (10) *Monkey*: 30% (9); 94% (49); 100% (24) *Cat*: 63% (13); 80% (15); 100% (25) *Rat*: 50% (40); 13.5% layer II/III, 100% layers V/VI (33); 97% (19); 100% layer V (26)		
CR	*Human*: 74% layers II–IIIA (10) *Monkey*: 91% (37) *Rat*: 25% (40); 94% (19); 100% (33) *Mouse*: 93% (47)		

Abbreviations (*continued*)

CB: calbindin
CCK: cholecystokinin
CR: calretinin
ChAT: choline acetyltransferase
GABA: gamma-aminobutyric acid
GAD: glutamic acid decarboxylase
nNOS: nitric oxide synthase
NPY: neuropeptide Y
PV: parvalbumin
SOM: somatostatin
TH: tyrosine hydroxylase
VIP: vasoactive intestinal polypeptide

region of pyramidal cells are GABAergic, while the glutamatergic axon terminals establish synapses with the adjacent dendrites. Consequently, any interneuron forming synapses in the perisomatic region of the pyramidal cells must be GABAergic. Therefore, chandelier cells and the variety of interneurons that form synapses with the somata (ASD cells and ADS cells), are by definition

Table 15.1 *continued*

	CCK	**NPY**	**VIP**
GABA/GAD	*Cat*: 11% (13)	*Cat*: 1% (13)	*Cat*: 0% (13)
ChAT			*Rat*: 32% (7); 82% (17); 97% (46)
TH			
CCK		*Cat*: 0% (13) *Rat*: 0% (34, 38)	*Cat*: 0% (13) *Rat*: 26% layer VI, 51% layer V, 63% layers II/III (34)
NPY	*Cat*: 0% (13) *Rat*: 0% (34, 38)		*Cat*: 0% (13) *Rat*: 0% (38)
VIP	*Cat*: 0% (13) *Rat*: 10% layer VI, 12% layer V, 24% layers II/III (34)	*Cat*: 0% (13)	
SOM	*Cat*: 0% (13, 44) *Rat*: 0% (34)	*Human*: 90% (31) *Monkey*: 41% (23); 80% (21) *Cat*: 80% (21) *Rat*: 33% (27); 42% layers I/III, 34% layers V/VI (33)	*Cat*: 0% (13) *Rat*: 0% (43)
nNOS	*Rat*: 0% (34)	*Monkey*: 99% large cells, 22% small cells (43) *Rat*: 100% (33)	
PV	*Cat*: 0% (15) *Rat*: 0% (27, 29, 34)	*Cat*: 0% (15) *Rat*: 0% (33)	*Rat*: 0% (27, 33)
CB	*Cat*: 0% (13, 15) *Rat*: 0–1% (34)	*Cat*: 0% (13, 15) *Rat*: 39% (33)	*Cat*: 0% (13) *Rat*: 0% (33, 40)
CR	*Rat*: 2% layers II/III, 3% layer VI, 5% layer V (34)	*Rat*: 0% (33)	*Monkey*: 86% (18) *Rat*: 71% layers II/III, 94% layers V/VI (33, 27); 90% (40); 63–94% (46)

References:

(1) Adams et al. 1993
(2) Alcantara et al. 1996
(3) Bayraktar et al. 1997
(4) Benavides-Piccione and DeFelipe 2003
(5) Carder et al. 1996
(6) Celio 1986
(7) Chédotal et al. 1994
(8) Condé et al. 1994
(9) DeFelipe and Jones 1992
(10) Del Rio and DeFelipe 1996
(11) Del Rio and DeFelipe 1997a
(12) Del Rio and DeFelipe 1997b
(13) Demeulemeester et al. 1988
(14) Demeulemeester et al. 1989
(15) Demeulemeester et al. 1991
(16) Dun et al. 1994
(17) Eckenstein and Baughman 1984
(18) Gabbott and Bacon 1997
(19) Gonchar and Burkhalter 1997
(20) González-Albo et al. 2001
(21) Jones and Hendry 1986
(22) Hendry et al. 1984a
(23) Hendry et al. 1984b
(24) Hendry et al. 1989

Table 15.1 *continued*

	PV	CB	CR
GABA/ GAD	*Monkey*: 74% (49) *Cat*: 37% (15); 50–60% (25) *Rat*: 50% (19); 54% (39); 70% (6) *Mouse*: 40% (47)	*Human*: 33% (10) *Monkey*: 12% (49) *Cat*: 10–15% (25); 18% (15); 20% (14) *Rat*: 19% (19)	*Human*: 46% layers II–IIIA (10) *Rat*: 17% (19) *Mouse*: 14% (47)
ChAT			
TH			
CCK	*Cat*: 0% (15) *Rat*: 0% (29, 34)	*Cat*: 0% (13, 15) *Rat*: 0–1% layers II/III, 8% layers V/VI (34)	*Rat*: 5% layers II/III, 11% layer VI, 25% layer V (34)
NPY	*Cat*: 0% (15)	*Cat*: 0% (11, 13) *Rat*: 68% layers II/III, 95% layers V/VI (33)	
VIP		*Cat*: 0% (13) *Rat*: 0% (40)	*Monkey*: 81% (18) *Rat*: 57% layers II/III, 94% layers V/VI (33); 95% (40); 45–95% (46)
SOM	*Human*: 0% (20) *Rat*: 0–0.5% (19, 29, 45)	*Human*: 82% (20) *Rat*: 75% layers II/III, 84% layers V/VI (40); 85% layers II/III, 92% layers V/VI (27, 33); 86% (19)	*Human*: 0% (20) *Rat*: 0% (19, 40)
nNOS	*Human*: 0% (20) *Monkey*: 0% (43) *Rat*: 0% (19); 0.23% (16)	*Human*: 40% (20) *Monkey*: 87–98% small cells, 0% large cells (43) *Rat*: 1.4% (16); 29% layers II/III, 80% layers V/VI (33); 64% (19)	*Human*: 0% (20) *Monkey*: 0% (43) *Rat*: 0% (19); 0.2% (16)
PV		*Human*: 0% (28); 4% (1); 14% (11) *Monkey*: 0% (8, 50); 0–1% (24) *Cat*: 0% (13, 14, 15, 25) *Rat*: 2.5% layer V (26); 5% infragranular layers (32); 5.3% (19); 91% layers II/III, 6% layers V/VI (27); 91% layers II/III (33); 21% layers II/III, 10% layer IV, <3% layers V/VI (2)	*Human*: 3% (35) *Monkey*: 0–1% (8, 37, 50) *Cat*: 0% (42) *Rat*: 0% (19, 33)
CB	*Human*: 0% (28); 5% (1); 9% (11) *Monkey*: 0% (2, 8, 50); <1% (24) *Cat*: 0% (14, 15, 25) *Rat*: 2.5% layer V (26); 12% (19); 14% infragranular layers (32)		*Human*: 6.2% (10); 10.6% layers II-IIIA (12) *Monkey*: 0% (8, 37, 50) *Cat*: 0% (42) *Rat*: 0% (19, 41)
CR	*Monkey*: 0% (8, 50); 0.9% (37) *Cat*: 0% (42) *Rat*: 0% (19, 27)	*Human*: 4.4% (10); 7–7.4% (35); 11% (12) *Monkey*: 0% (8, 37, 50) *Cat*: 0% (42) *Rat*: 0% (19, 27, 41); 6% (33)	

(*continued*)

Table 15.1 *continued*

	SOM	**nNOS**
GABA/ GAD	*Cat*: 2% (13) *Rat*: 17% (19) *Mouse*: 23.4% (47)	*Rat*: 0.5% (19)
ChAT		
TH		*Human*: 25% layers V–VI (4)
CCK	*Cat*: 0% (13, 44) *Rat*: 0% (34)	*Rat*: 0% (34)
NPY	*Human*: 90% (31) Monkey: 37% (23) *Rat*: 71% layers II/III, 90% layers V/VI (33)	*Rat*: 43% layers II/III, 52% layers V/VI (33)
VIP	*Cat*: 0% (13) *Rat*: 0% (33)	
SOM		*Monkey*: 25–30% (43) *Rat*: 1.7% (19); 11% layers II/III, 26% layers V/VI (33)
nNOS	*Monkey*: 99% large cells, <5% small cells (43) *Rat*: 100% (19, 33)	
PV	*Human*: 0% (layers II/III, 20) *Cat*: 0% (15) *Rat*: 0–0.2% (19, 27, 29, 33, 45)	*Human*: 0% layers II/III (20) *Monkey*: 18–22% (43) *Rat*: 0% (19, 33)
CB	*Human*: 44% layers II/III (20) *Cat*: 7% (13, 15) *Rat*: 68% (33); 70% (19); 75% layers II/III, 84% layers V/VI (40)	*Human*: 25% (layers II/III, 20) *Rat*: 1.2% (19); 8% (33)
CR	*Human*: 0% layers II/III (20) *Rat*: 0% (19, 27, 33, 40)	*Human*: 0% layers II/III (20) *Rat*: 0% (19, 33)

References

(25) Hendry and Jones 1991
(26) Kawaguchi and Kubota 1993
(27) Kawaguchi and Kubota 1997
(28) Kobayashi et al. 1990
(29) Kosaka et al. 1987
(30) Kosaka et al. 1988
(31) Kowall and Beal 1988
(32) Kubota and Jones 1993
(33) Kubota et al. 1994
(34) Kubota and Kawaguchi 1997
(35) Leuba and Saini 1997
(36) Lin et al. 1986
(37) Meskenaite 1997
(38) Papadopoulos et al. 1987
(39) Ren et al. 1992
(40) Rogers 1992
(41) Rogers and Resibois 1992
(42) Schwark and Li 2000
(43) Smiley et al. 2000
(44) Somogyi et al. 1984
(45) Standaert et al. 1996
(46) Taki et al. 2000
(47) Tamamaki et al. 2003
(48) Trottier et al. 1989
(49) van Brederode et al. 1990
(50) Vickers et al. 1993

GABAergic, whereas those interneurons that do not express GABA must establish synapses with the distal dendrites of pyramidal cells or with other interneurons.

Immunocytochemistry for CB, PV, and CR has proved to be a useful tool for the biochemical characterization of certain interneurons (reviewed in Andressen et al. 1993; DeFelipe 1993, 1997). These three calcium-binding proteins show little or no co-localization (Table 15.1), and with some exceptions, each of them labels distinct types of interneurons. For example, double bouquet cells can be labeled for CB or CR, but never for PV, while bipolar cells contain CR. PV is found in chandelier cells, although a subpopulation in layers V–VI contain CB. However, chandelier terminals are never labeled with CR. Moreover, a good deal of information can be obtained from double-labeling experiments involving calcium-binding proteins and the detection of other substances. For instance, the fact that neurons containing PV do not express CCK, NPY, SOM, and nNOS (Table 15.1) indicates that chandelier cells do not express these substances. A major conclusion from such double-labeling studies, and from those using intracellular recording in combination with anatomical and immunocytochemistry studies, or combining electrophysiology with *in situ* hybridization or PCR techniques, is that, in general, different types of interneurons are characterized by a particular combination of molecular, physiological, and synaptic features (Cauli et al. 1997; Kawaguchi and Kubota 1997; Silberberg et al. 2002 Kawaguchi and Kondo 2002).

LAMINAR DISTRIBUTION AND INTERNEURON TYPES FREQUENCY

Immunocytochemistry also provides a good way to estimate the laminar distribution and frequency of the various types of interneurons (e.g., Condé et al. 1994; Gonchar and Burkhalter 1997; Gabbott and Bacon 1996; González-Albo et al. 2001). For example, in layer I of the human temporal cortex (González-Albo et al. 2001), no or very few neurons can be stained for nNOS, SOM, NPY, or PV, whereas neurons expressing CB and CR can be found in this layer. Neurons expressing nNOS and NPY are distributed relatively homogeneously in layers II–VI, whereas those containing SOM, PV, CB, and CR are more abundant in layers II–III. Immunocytochemical studies have also shown considerable variation in the density of neurons that contain these substances in the human cortex. For example, the density of neurons stained for CR is 61% higher than that of neurons stained for nNOS, and 88% higher than that of neurons stained for NPY (González-Albo et al. 2001). These observations suggest that each of these subpopulations of interneurons contribute differently to inhibitory circuits, in a laminar-specific manner. Furthermore, GABAergic cells constitute 15% of the total population of neurons in all cortical areas of the rat (e.g.,

Beaulieu 1993), whereas in the primate, they represent 20 % in the visual cortex and up to 25% in other cortical areas (e.g., Hendry et al. 1987). Importantly, Beaulieu (1993) pointed out that the same proportion of GABAergic neurons (15%) was found in the occipital, parietal, and frontal cortex of the rat, while the density of total neurons in the occipital and parietal cortex was approximately 1.5 times greater than in the frontal cortex. Similar observations have also been made in the macaque cortex, and indeed area 17 of the macaque monkey has more than twice the total density of neurons when compared to other cortical areas (including areas 4, 3b, 1, 2, 5, 7, 18, 21, and areas of the orbital frontal and lateral frontal cortex; see Hendry et al. 1987). However, the differences in the proportion of GABAergic cells between area 17 and the other cortical areas examined in the macaque were never greater than 5%. These findings suggest that the proportion of interneurons is relatively fixed in different species, regardless of the number of neurons. Nevertheless, this does not mean that the different types of interneurons are equally represented in all the cortical areas. In fact, several lines of evidence indicate the contrary. For example, the number and distribution of double bouquet cells and chandelier cells differs considerably between different areas of the occipital (areas 17 and 18) and temporal (area TE) lobes of the macaque monkey. This strongly suggests that regional specialization of inhibitory circuits occurs (DeFelipe et al. 1999).

Finally, it is important to note that not all types of interneurons are found in all species and that the molecular characteristics of interneurons may differ between species. Furthermore, there are significant variations between species in the number of symmetrical (inhibitory) synapses per neuron, which indicates important differences in cortical circuits at the synaptic level (DeFelipe et al. 2002). Therefore, caution should be taken when extrapolating data on interneuronal circuits obtained in any given species to all mammalian species.

MOLECULAR DIVERSITY OF INTERNEURONS

The morphological and neurochemical diversity of GABAergic interneuron subtypes is accompanied by an individual repertoire of receptors and ion channels, which is of major functional importance at the cellular and network level. Because of the relatively simple and structured architecture of the hippocampus, GABAergic interneurons have been mostly studied and classified in this structure, rather than in the neocortex. Hippocampal classification criteria have been often extended to “analogous” cell types in the neocortex, even though the neurochemical and physiological signature of neocortical interneurons does not always match the postsynaptic target distribution as precisely as in the hippocampus. Most importantly, for the accurate classification/characterization of interneuronal cell types, anatomical features and molecular–functional properties should be investigated using a systematic and combinatorial approach.

Molecular Techniques

Different anatomical and electrophysiological techniques used over the past twenty years to study channel and receptor heterogeneity in the brain and which contributed to revealing molecular diversity are mentioned below. Furthermore, single-cell RT-PCR proved to be a particularly valuable technique to correlate functional with molecular data. Technical details, however, are beyond the scope of this chapter, but an attempt is made to mention the pertinent references wherever possible for the interested reader.

Expression studies entailed mainly *in situ* hybridization and immunocytochemical studies. *In situ* hybridization studies are carried out using either riboprobes or short oligonucleotide probes. Riboprobes have the advantage that they permit double-labeling, which has not been possible so far when using oligonucleotide probes. Double-labeling experiments can be performed either using two different riboprobes (e.g., one radioactive-labeled and one nonradioactive-labeled probe) or using a riboprobe and an antibody. The clear disadvantage of riboprobes is the propensity of nonspecific labeling of brain structures with high neuronal density (particularly pyramidal cell layer of the hippocampus and granule cell layer of the cerebellum). Oligonucleotide probes are definitely more reliable in this respect since nonspecific labeling can clearly be detected when a signal is present in competition experiments using an excess of nonlabeled oligoprobe. The complementary use of both techniques is advisable: first establish the "correct" expression pattern using an oligonucleotide probe, and then establish the optimal "working conditions" for the riboprobe (Laurie et al. 2002). Immunocytochemistry using specific antibodies allows the expression analysis at the protein level. Furthermore, co-localization of several proteins can be studied with multiple-labeling techniques. In addition to detecting whether a protein is expressed in GABAergic interneurons or not, the availability of antibodies also permits studies regarding the cellular compartment (Kanai and Hirokawa 1995; Waldvogel et al. 1999). In particular, electron microscopy techniques allow to discern the subcellular localization of proteins at the ultrastructural level.

Molecular cloning permitted functional studies of recombinant receptors in different expression systems (oocytes, cell lines). Studies in heterologous expression systems gave first hints as to likely subunit composition in different brain regions and sometimes even cell types. Thus, in conjunction with *in situ* hybridization studies, functional diversity of different receptors in subsets of neurons, including GABAergic cells, was predicted from electrophysiological characterization of recombinant nicotinic, $GABA_A$, or glutamate receptors (for review see Seeburg 1993; Levitan et al. 1988; Changeux et al. 1984). Sometimes, functional studies of recombinant receptors revealed channel properties that had been unknown based on previous studies of native receptors.

Functional studies revealing specific characteristics of GABAergic interneurons were most often electrophysiological studies. The availability of the different cloned channels permitted numerous investigations: whole-cell recordings were helpful as initial screening tests for channel functionality whereas measurements from outside-out patches, particularly in conjunction with fast agonist application, were useful in studies of channel kinetics.

A molecular technique that has been used frequently for mRNA expression analysis in GABAergic interneurons is single-cell RT-PCR. There are reports on the use of one set of primers to amplify one or several mRNAs belonging to the same family or studies in which several sets of primers were employed to perform multiplex-PCR with the aim to detect a number of different unrelated mRNAs. Advantages and disadvantages of these methods have been discussed elsewhere (Monyer and Markram 2004); the choice should be dictated by the scientific question. It cannot be stressed often enough, however, that both approaches are most powerful when combined with functional studies.

With these tools at hand, a number of studies provided evidence that in many instances GABAergic interneurons express a different set of genes compared to their counterpart, the pyramidal cells. This is a simplified generalization, since we have just begun to understand that the above described anatomical diversity is most likely matched by a molecular diversity. In many instances, this molecular diversity is inferred based on data obtained in the hippocampus, a structure that at least with respect to certain aspects is better studied than the neocortex.

Let us consider an example: molecular cloning of the glutamate receptors of the AMPA class revealed the existence of four subunits, GluR-A to D (or 1 to 4), each having two splice variants termed "flip" and "flop" (Keinanen et al. 1990). *In situ* hybridization studies using oligonucleotide probes clearly indicated differential distribution in the brain and even permitted the prediction that certain GABAergic interneurons (e.g., in stratum radiatum and moleculare of the hippocampus) may express little GluR-B and that the expressed subunits GluR-A and GluR-D are mainly in the "flop" splice version (Monyer et al. 1991). This prediction was confirmed by electrophysiological studies combined with single-cell RT-PCR (Geiger et al. 1995). Double-labeling studies (Catania et al. 1995; Leranth et al. 1996) using a riboprobe for the GluRs and an antibody to label GABAergic interneurons could ascertain the hypothesis of low GluR-B expression in most GABAergic interneurons. In addition, the expression of AMPA receptors was analyzed using specific GluR antibodies (Wenthold et al. 1992).

Receptor Diversity

Our knowledge about cortical GABAergic interneuron diversity based on molecular criteria is patchy at best, but a brief summary will also indicate the need of further studies in this field.

AMPA Receptors

The existence of several subunits and splice variants for the three major ionotropic glutamate receptor subfamilies (AMPA, NMDA, and kainate) prompted investigations regarding their expression in GABAergic interneurons. Single-cell RT-PCR combined with outside-out patch measurements permitted correlative studies indicating that low abundance of GluR-B accounted for the high Ca^{2+} permeability of AMPA receptors in fast-spiking (FS) GABAergic interneurons in somatosensory (Hestrin 1993) and visual cortex (Jonas et al. 1994). In contrast to this specific property of GABAergic interneurons, for which one single molecular determinant appears to be responsible, kinetic characteristics of AMPA receptors in GABAergic interneurons are regulated by several molecular mechanisms. In subsequent studies, the same technical approach led to the conclusion that in addition to subunit composition, splicing and editing also contribute to determine the faster channel kinetics (Geiger et al. 1995; Lambolez et al. 1996; Angulo et al. 1997). Although the generalization regarding low GluR-B expression in GABAergic interneurons in the cortex is justified, differences in AMPA receptor Ca^{2+} permeability and conductance in different GABAergic subtypes have been reported. Thus, in contrast to FS PV-positive interneurons characterized by AMPA receptors with high Ca^{2+} permeability, a number of other GABAergic interneuron subtypes bear AMPA receptors with lower Ca^{2+} permeability. These interneurons comprise VIP-positive bitufted interneurons (Rozov et al. 2001) or CB- and PV-positive multipolar bursting (MB) cells (Blatow et al. 2003) in cortical layer II/III (Figure 15.3). Also, desensitization and recovery from desensitization exhibit marked differences in identified subsets of GABAergic interneurons (Angulo et al. 1997; Rozov et al. 2001). The existing examples permit at this point the following conclusion: in spite of some variability between different GABAergic interneuron subtypes, lower GluR-B expression than in pyramidal neurons appears to be a hallmark of AMPA receptor characteristics in this cell population. The differences between GABAergic interneuron subtypes are more pronounced when kinetic properties of AMPA receptors are considered. Although interesting at a phenomenological level, these observations lead to the intriguing question: What is the functional significance of AMPA receptor diversity on GABAergic interneurons at the network level? Making use of a transgenic approach helped in resolving this question not in the neocortex but in the hippocampus. Selective overexpression of the GluR-B subunit in GABAergic interneurons indicated that AMPA receptor properties in this type of cells subserve long-range synchrony (Fuchs et al. 2001). Another elegant study merits emphasis in this context: Rozov and colleagues (2001) could show that kinetic properties of AMPA receptors on VIP-positive bitufted interneurons in layer II/III of rat somatosensory cortex accounted for the frequency-dependent depressing responses (EPSPs and EPSCs) elicited in these cells upon repetitive stimulation of pyramidal neurons. Fast

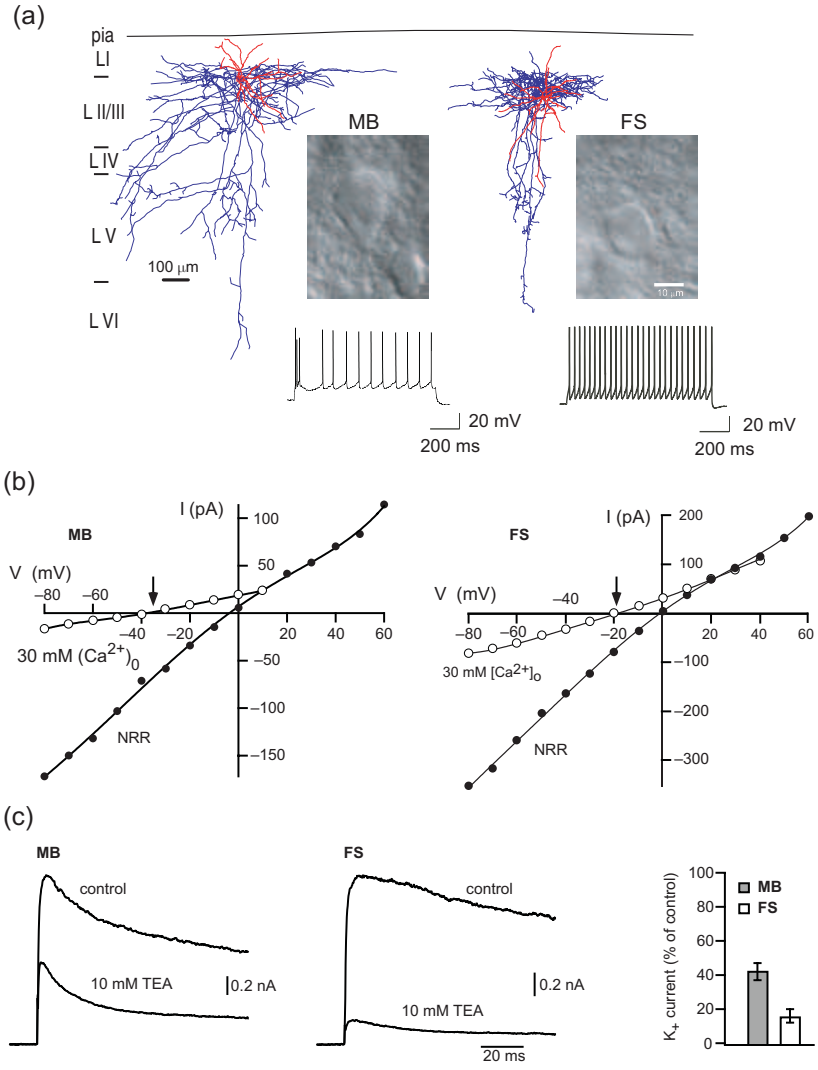

Figure 15.3 Anatomical and molecular diversity of cortical interneurons exemplified by multipolar bursting (MB) and fast-spiking (FS) cells that constitute two distinct subpopulations of parvalbumin (PV)-positive cortical cells. (a) Representative reconstructions of the dendritic (red) and axonal (blue) arbors of a biocytin-filled MB cell (left) and an FS cell (right) in layer II/III of mouse neocortex. Representative IR–DIC images and typical firing patterns of action potentials (APs) upon depolarizing current injection are shown in the insets for both cell types. (b) Properties of somatic AMPARs expressed in MB cells and FS cells. The Ca^{2+} permeability of AMPARs is shown in the current–voltage relationships for glutamate-evoked currents recorded from nucleated patches pulled from MB cells (left) and FS cells (right) in normal rat Ringer's (NRR; closed circles) and high Ca^{2+} (30 mM $(Ca^{2+})_0$; open circles) solutions. *Caption continues on next page*.

desensitization and slow recovery from desensitization of AMPA receptors on these GABAergic interneurons enable these cells to contribute to low-pass feedback inhibition of pyramidal neurons.

NMDA Receptors

Studies on NMDA receptor expression and their functional implication in cortical GABAergic interneurons are rare. In analogy to the hippocampus, it is probably safe to assume that cortical GABAergic interneurons express also NR2A and NR2B, alone or in combination (Monyer et al. 1994). The finding of the preferential expression of the NR2D subunit in SOM- and PV-positive interneurons is based on an *in situ* hybridization study in different brain regions, including the neocortex (Standaert et al. 1996). Of note is that in the neocortex, SOM-positive cells exhibit higher NR2D expression compared to PV-positive neurons. However, the functional role of this subunit in these GABAergic interneurons is not clear. Although the long deactivation time constant (more than 4 seconds) of NR2D-containing recombinant receptors suggests a possible function for integration of presynaptic input over longer periods, this hypothesis remains speculative since such long NMDA receptor deactivation kinetics have not been found in neurons. In contrast to pyramidal neurons where it is known that both NR2A and NR2B are synaptically expressed, there is no information about the subcellular localization of NR2A, B, or D in GABAergic interneurons. Based on the peculiarity of glutamate receptor subunit expression, both NMDA and AMPA receptors can contribute to Ca^{2+} entry in GABAergic interneurons, hence the question as to the relative contribution and functional role of these two glutamate receptor-mediated Ca^{2+} entry sources. Ca^{2+} imaging studies in different types of layer II mouse visual cortex interneurons indicate that Ca^{2+}-permeable AMPA and NMDA receptors differentially contribute to subthreshold Ca^{2+} dynamics in distinct GABAergic interneuron subtypes. Although dendritic Ca^{2+} influx through NMDA receptors is functionally important in PV-positive FS and CR-positive irregular spiking cells, synaptic Ca^{2+} influx through AMPA receptors is functionally relevant only in FS cells contributing to a larger peak and faster Ca^{2+} kinetics in this cell type (Goldberg et al. 2003).

Figure 15.3 (*continued*) Arrows indicate Ca^{2+}/Cs^{2+} reverse potentials. The Ca^{2+}/Cs^{2+} reverse potential in MB cells is more negative, indicating a lower Ca^{2+} permeability of AMPARs than in FS cells. (c) Properties of voltage-gated K^+ channels expressed in MB cells and FS cells. Example traces show the effect of 10 mM TEA on K^+ currents measured at +80 mV following a pre-pulse to –110 mV in nucleated patches from MB cells and FS cells. The reduction of current amplitude upon TEA application is less pronounced in MB cells, indicating a lower expression of TEA-sensitive K^+ channels. The histogram on the right shows the average reduction of current amplitude. These experiments were done in the presence of 100 nM TTX to block voltage-gated Na^+ channels.

Metabotropic Glutamate Receptors

Expression of metabotropic glutamate receptors (mGluRs) in cortical interneurons has obtained little attention so far. Only one study investigating mGluR1a expression concluded that most mGluR1a-positive cells in the neocortex were GABAergic interneurons (Stinehelfer et al. 2000). Although purely descriptive (being an *in situ* hybridization study), these results revealed not only differential expression between different GABAergic interneuron subclasses but also within a class. There was no homogeneity of expression of mGluR1a even within one subclass as judged by double-labeling for biochemical markers: most, but not all SOM-positive neurons, about 50% of CR-positive cells, 30% of CB-positive cells, and almost no PV-positive cells expressed mGluR1a.

$GABA_A$ Receptors

The cloning of the first ionotropic $GABA_A$ receptor subunits was done in the early 1980s and the paucity of information regarding their differential expression in GABAergic interneurons is surprising. This is in stark contrast to the growing body of evidence that $GABA_A$ receptors on GABAergic interneurons are also a key requirement for the generation of oscillatory activity in different frequency ranges (Whittington et al. 1995). For a GABAergic interneuron network formed of low threshold spiking (LTS) cells in layer IV of somatosensory cortex, it was shown that activation of $GABA_A$ receptors was necessary for the induction of theta frequency oscillations, (Gibson et al. 1999). In another network of chemically and electrically coupled MB cells (Blatow et al. 2003) in frontal and somatosensory cortex layer II/III, theta frequency oscillations also depended on interneuron $GABA_A$ conductance. However, little is known about the exact $GABA_A$ receptor subunit composition in the different GABAergic cell populations. In a recent study performed in layer V of the somatosensory cortex, Bacci et al. (2003b) came to the conclusion that functional differences in spontaneous and induced IPSCs recorded in FS and LTS cells can be accounted for at least in part by differential expression of the $GABA_A$ receptor subunits. The higher expression of $\alpha 1$ as well as $\beta 2$–3 subunits in FS cells may underlie the higher amplitude and faster kinetics of IPSCs in these GABAergic interneurons. The authors used pharmacological tools and knockout animals to correlate IPSC waveform with differential $GABA_A$ receptor subunit expression in FS and LTS cells. Faster kinetics of IPSCs have also been reported for FS PV-positive interneurons in the hippocampus (Bartos et al. 2002), suggesting that fast IPSCs may be a feature that is shared by FS cells in hippocampus and neocortex.

5-HT_3 and Nicotinic Receptors

A less confusing picture emerged upon studying 5-HT_3R in neocortex. *In situ* hybridization and immunocytochemistry (Morales et al. 1996; Morales and

Bloom 1997) results are in agreement with data obtained using RT-PCR (Ferezou et al. 2002). The 5-HT_3R, the only ionotropic 5-HT receptor, is expressed in a subset of GABAergic interneurons, namely, the CCK/VIP-positive neurons. This rule appears to hold for all cortical layers. Interestingly, in the RT-PCR study, the authors reported co-expression of 5-HT_3 and nicotinic receptors in CCK/VIP-positive GABAergic interneurons. The nicotinic receptors mediating the excitatory effect are presumably composed of the α4, α5, and β2 subunits as indicated by previous pharmacological and RT-PCR results (Porter et al. 1999). This and other studies on nicotinic receptors are of note because they clearly indicate that caution needs to be exerted when extrapolating data and drawing conclusions based on findings from one brain region in a species. Thus, in the rat neocortex, nicotinic receptors are selectively expressed in CCK/VIP-positive interneurons and are not present in pyramidal neurons. However, the restricted expression of nicotinic receptors to a subset of GABAergic interneurons may differ between species, since in the developing visual cortex there is functional evidence for their presence on pyramidal neurons as well (Roerig et al. 1997). Also, it appears that nicotinic receptors in interneurons of the hippocampus may not be restricted to CCK/VIP-positive neurons and, in addition, the nicotinic receptor subunit composition appears to be different in hippocampal and neocortical interneurons.

Other Receptors

GABAergic interneuron diversity is also manifested in the differential expression of μ opioid receptor in a subset of GABAergic interneurons in the cortex (Taki et al. 2000). Although absent from interneurons expressing PV or CB, μ-opioid receptor expression was found to a varying degree in a nonhomogeneous interneuron population, expressing any of the following markers: VIP, CRF, ChAT, CR, or CCK.

Although not specifically expressed in GABAergic interneurons but also in pyramidal neurons, expression analysis of D_1 and D_2 dopamine receptor subunits indicates that both receptors are more often found in PV-positive compared to CB-positive neurons in the neocortex (Le Moine and Gaspar 1998). Functional implications are not clear at a cellular level, let alone at a network level. The study is mentioned here for completion's sake. Similarly, expression of the cannabinoid receptor CB1 is not restricted to GABAergic interneurons in most brain areas. Activation of CB1 receptors exerts modulatory effects onto glutamatergic and GABAergic neurons. However, in the neocortex, at least in rat and mouse brain, there is an excellent correlation between CB1 expression and GABAergic interneurons. Expression diversity within the GABAergic system is illustrated best by mentioning the two extremes: CCK-positive neurons exhibit the highest expression levels; PV- and CR-positive cells are CB1-negative (Matsuda et al. 1993; Tsou et al. 1998; Marsicano and Lutz 1999).

The above-mentioned studies clearly revealed that a number of modulatory effects mediated by nicotinic, 5-HT_3, or CB1 receptors are exerted both in the neocortex as well as in the hippocampus exclusively or preponderantly on the GABAergic system.

Potassium Channels

Differential expression of voltage-gated channels in neocortex is best studied for K^+ channels. *In situ* hybridization experiments indicate that in the neocortex the expression of the four K^+ channels (Kv3.1, Kv3.2, Kv3.3, and Kv3.4) is restricted to GABAergic interneurons (Weiser et al. 1994). Layer-specific expression and differential expression levels were indications of combinatorial possibilities of heteromeric assemblies. Subsequent analysis concentrated on the fast activation/deactivation channels Kv3.1 and Kv3.2 that, based on functional analysis in oocytes, were thought to be the molecular substrate for fast spiking. Cellular distribution analysis revealed the presence of Kv3.1 in PV-positive cells in all cortical layers whereas Kv3.2 was found mainly in deeper cortical layers. Since the latter was not only detected in PV-positive neurons but also in SOM- and CB-positive cells, a simple correlation between Kv3.1 and/or Kv3.2 and fast spiking could not be made. In fact, a study supports the notion that, at least in the hippocampus, the combination of Kv3.1 and Kv3.4 underlies fast spiking in PV-positive interneurons (Baranauskas et al. 2003). The functional significance at a network level of these K^+ channels, which are prominent in the GABAergic system, is apparent in Kv3.1 KO mice and manifests itself in altered oscillatory activity (increased gamma and decreased delta power; Joho et al. 1999). These findings support the notion that GABAergic interneurons are critically involved in the generation of synchronous, oscillatory network activity (for a review, see McBain and Fisahn 2001).

Gap Junction-forming Proteins

An interesting example for differential expression of a protein in GABAergic interneurons and its functional implication at the network level is the gap junction-forming protein connexin 36 (Cx36). In the neocortex, Cx36 is preferentially expressed in GABAergic interneurons as documented by *in situ* hybridization and RT-PCR. Most of the PV-positive cell population in all cortical layers expresses Cx36 (Belluardo et al. 2000). It is certainly the molecular substrate of gap junction coupling as reported for LTS (Deans et al. 2001), FS (Hormuzdi et al. 2001), and MB cells (Blatow et al. 2003). Functional evidence for gap junction coupling in SOM-positive layer IV LTS cells (Gibson et al. 1999) and in SOM-positive layer II/III bipolar neurons (Venance et al. 2000), indicates that gap junction coupling is not a prerogative of PV-positive neurons. However,

only interneurons of the same subtype seem to be electrically coupled (Gibson et al. 1999; Meyer et al. 2002; Blatow et al. 2003). The segregation of electrically coupled networks appears to be very strict indeed, since the expression of Cx36 does not imply functional gap junctions between different neurons. As a striking example two subsets of PV- and Cx36-expressing neurons, FS cells and MB cells, belong to distinct networks each delineated by electrical coupling amongst neurons of the same subtype (Blatow et al. 2003). Although PV-positive, these two cell types were different with respect to most criteria that were studied (see Figure 15.3). More recently, we have characterized two members of a new gap junction-forming protein family, the pannexins (Pxs) (Bruzzone et al. 2003). Albeit not selectively expressed in GABAergic interneurons, mRNA expression levels of Px1 and Px2 are higher in neocortical (and hippocampal) GABAergic interneurons compared to pyramidal cells (Monyer, unpublished). Their presence in PV-positive interneurons (unpublished observation) raises the intriguing question as to whether they co-localize or not with Cx36 and, if so, to which cellular compartment the gap junction proteins are targeted.

PERSPECTIVES

Although often merely descriptive, the above-mentioned examples represent major contributions toward a better understanding of GABAergic interneuron diversity at a functional level. Information regarding the individual morphological, molecular, and functional characteristics of particular interneuron subtypes gathered thus far suggests that these cells subserve distinct roles in the frame of larger circuitries. Indeed, different interneuron subclasses (e.g., FS, LTS, and MB cells) appear to form discrete networks participating in various oscillation phenomena.

In a recent review, a number of techniques to study GABAergic interneuron diversity were discussed (Monyer and Markram 2004). It is certainly clear that GABAergic interneuron diversity on the one hand and the sparsity of certain subtypes on the other makes the functional characterization of identified GABAergic interneurons a daunting task. Significant progress in this field of research will be heralded by the following approaches: (a) combinatorial approaches using RT-PCR in conjunction with anatomical and functional characterization; (b) *in vivo* labeling of GABAergic subpopulations (see Figure 15.4); (c) selective knockouts of key molecules in GABAergic interneurons; (d) functional silencing of distinct GABAergic networks. These approaches will ultimately allow an assessment of the role of GABAergic cells in the living animal. To this end, a first attempt has been recently reported by Margrie et al. (2003), where genetically labeled PV-positive cells were patched *in vivo* using two-photon microscopy.

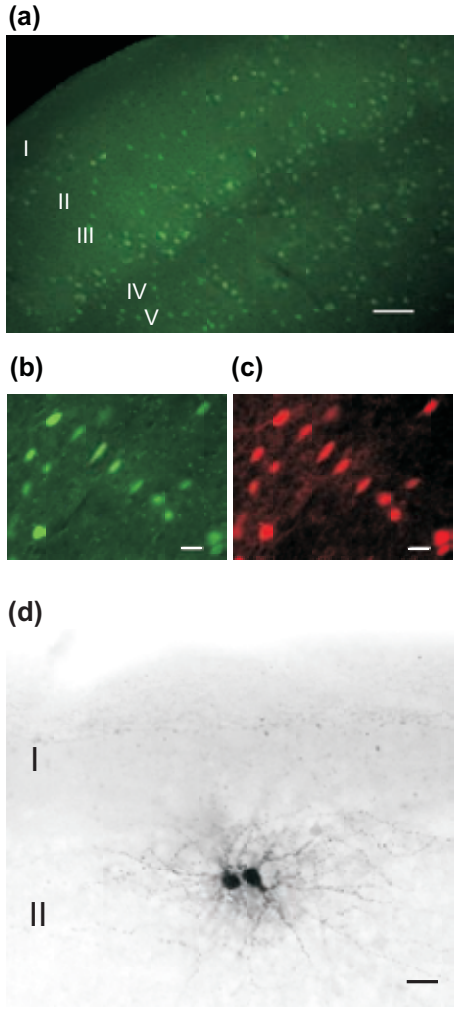

Figure 15.4 Transgenic techniques will aid in the analysis of distinct populations of cortical interneurons. Here the example is given of transgenic mice expressing the *in vivo* marker EGFP in PV-positive cells. (a) EGFP-labeled fluorescent cells are visible throughout the neocortex of the PV–EGFP transgenic mouse. EGFP expression (b) strongly co-localizes with PV expression (c) as confirmed by immunocytochemistry. (d) *In vivo* EGFP expression enables the identification of PV-positive cells in the acute slice preparation. After electrophysiological studies cells can be filled with biocytin and processed for morphological analysis. The example of two gap junction coupled FS cells in layer II/III is shown. Scale bars: (a) = 100 μm; (b)–(d) = 20 μm.

REFERENCES

Adams, L.A., L.-C. Ang, and D.G. Munoz. 1993. Chromogranin A, a soluble synaptic vesicle protein, is found in cortical neurons other than previously defined peptidergic neurons in the human neocortex. *Brain Res.* **602**:336–341.

Alcantara, S., L. de Lecea, J.A. Del Rio, I. Ferrer, and E. Soriano. 1996. Transient co-localization of parvalbumin and calbindin D28k in the postnatal cerebral cortex: Evidence for a phenotypic shift in developing nonpyramidal neurons. *Eur. J. Neurosci.* **8**:1329–1339.

Alonso-Nanclares, L., A. Minelli, M. Melone et al. 2004. Perisomatic glutamatergic axon terminals: A novel feature of cortical synaptology revealed by vesicular glutamate transporter 1 immunostaining. *Neuroscience* **123**:547–556.

Amitai, Y., J.R. Gibson, M. Beierlein et al. 2002. The spatial dimensions of electrically coupled networks of interneurons in the neocortex. *J. Neurosci.* **22**:4142–4152.

Andressen, C., I. Blümcke, and M.R. Celio. 1993. Calcium-binding proteins: Selective markers of nerve cells. *Cell Tiss. Res.* **271**:181–208.

Angulo, M.C., B. Lambolez, E. Audinat, S. Hestrin, and J. Rossier. 1997. Subunit composition, kinetic, and permeation properties of AMPA receptors in single neocortical non-pyramidal cells. *J. Neurosci.* **17**:6685–6696.

Bacci, A., J.R. Huguenard, and D.A. Prince. 2003a. Functional autaptic neurotransmission in fast-spiking interneurons: A novel form of feedback inhibition in the neocortex. *J. Neurosci.* **23**:859–866.

Bacci, A., U. Rudolph, J.R. Huguenard, and D.A. Prince. 2003b. Major differences in inhibitory synaptic transmission onto two neocortical interneuron subclasses. *J. Neurosci.* **23**:9664–9674.

Baranauskas, G., T. Tkatch, K. Nagata, J.Z. Yeh, and D.J. Surmeier. 2003. Kv3.4 subunits enhance the repolarizing efficiency of Kv3.1 channels in fast-spiking neurons. *Nat. Neurosci.* **6**:258–266.

Bartos, M., I. Vida, M. Frotscher et al. 2002. Fast synaptic inhibition promotes synchronized gamma oscillations in hippocampal interneuron networks. *PNAS* **99**:13,222–13,227.

Bayraktar, T., J.F. Staiger, L. Acsady et al. 1997. Co-localization of vasoactive intestinal polypeptide, γ-aminobutyric acid and choline acetyltransferase in neocortical interneurons of the adult rat. *Brain Res.* **757**:209–217.

Beaulieu, B.C. 1993. Numerical data on neocortical neurons in adult rat, with special reference to the GABA population. *Brain Res.* **609**:284–292.

Belluardo, N., G. Mudo, A. Trovato-Salinaro et al. 2000. Expression of connexin36 in the adult and developing rat brain. *Brain Res.* **865**:121–138.

Benavides-Piccione, R., and J. DeFelipe. 2003. Different populations of tyrosine-hydroxylase-immunoreactive neurons defined by differential expression of nitric oxide synthase in the human temporal cortex. *Cereb. Cortex* **13**:297–307.

Blatow, M., A. Rozov, I. Katona et al. 2003. A novel network of multipolar bursting interneurons generates theta frequency oscillations in neocortex. *Neuron* **38**:805–817.

Bruzzone, R., S.G. Hormuzdi, M.T. Barbe, A. Herb, and H. Monyer. 2003. Pannexins, a family of gap junction proteins expressed in brain. *PNAS* **100**:13,644–13,649.

Cajal, S.R. 1904. Textura del sistema nervioso del hombre y de los vertebrados. Madrid: Moya.

Carder, R.K., S.S. Leclerc, and S.H.C. Hendry. 1996. Regulation of calcium-binding protein immunoreactivity in GABA neurons of macaque primary visual cortex. *Cereb. Cortex* **6**:271–287.

Catania, M.V., T.R. Tolle, and H. Monyer. 1995. Differential expression of AMPA receptor subunits in NOS-positive neurons of cortex, striatum, and hippocampus. *J. Neurosci.* **15**:7046–7061.

Cauli, B., E. Audinat, B. Lambolez et al. 1997. Molecular and physiological diversity of cortical non-pyramidal cells. *J. Neurosci.* **17**:3894–3906.

Celio, M.R. 1986. Parvalbumin in most gamma-aminobutyric acid containing neurones of the rat cerebral cortex. *Science* **231**:995–997.

Changeux, J.-P., A. Devillers-Thiery, and P. Chemouilli. 1984. Acetylcholine receptor: An allosteric protein. *Science* **225**:1335–1345.

Chédotal, A., C. Cozzani, M.-P. Faure, B.K. Hartman, and E. Hamel. 1994. Distinct choline acetyltransferase (ChAT) and vasoactive intestinal polypeptide (VIP) bipolar neurons project to local blood vessels in the rat cerebral cortex. *Brain Res.* **646**: 181–193.

Condé, F., J. Lund, D.M. Jacobowitz, K.G. Baimbridge, and D.A. Lewis. 1994. Local circuit neurones immunoreactive for calretinin, calbindin D-28k, or parvalbumin in monkey prefrontal cortex: Distribution and morphology. *J. Comp. Neurol.* **341**: 95–116.

Deans, M.R., J.R. Gibson, C. Sellitto, B.W. Connors, and D.L. Paul. 2001. Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing connexin36. *Neuron* **31**:477–485.

DeFelipe, J. 1993. Neocortical neuronal diversity: Chemical heterogeneity revealed by co-localization studies of classic neurotransmitters, neuropeptides, calcium binding proteins, and cell surface molecules. *Cereb. Cortex* **3**:273–289.

DeFelipe, J. 1997. Types of neurones, synaptic connections, and chemical characteristics of cells immunoreactive for calbindin-D28K, parvalbumin, and calretinin in the neocortex. *J. Chem. Neuroanat.* **14**:1–19.

DeFelipe, J. 2002. Cortical interneurons: From Cajal to 2001. *Prog. Brain Res.* **136**: 215–238.

DeFelipe, J., L. Alonso-Nanclares, and J.I. Arellano. 2002. Microstructure of the neocortex: Comparative aspects. *J. Neurocytol.* **31**:299–316.

DeFelipe, J., M.C. González-Albo, M.R. del Río, and G.N. Elston. 1999. Distribution and patterns of connectivity of interneurons containing calbindin, calretinin, and parvalbumin in visual areas of the occipital and temporal lobes of the macaque monkey. *J. Comp. Neurol.* **412**:515–526.

DeFelipe, J., S.H.C. Hendry, and E.G. Jones. 1986. A correlative electron microscopic study of basket cells and large GABAergic neurons in the monkey sensory-motor cortex. *Neuroscience* **7**:991–1009.

DeFelipe, J., and E.G. Jones. 1988. Cajal on the Cerebral Cortex. New York: Oxford Univ. Press.

DeFelipe, J., and E.G. Jones. 1992. High resolution light and electron microscopic immunocytochemistry of co-localized GABA and calbindin D-28K in somata of double-bouquet cell axons of monkey somatosensory cortex. *Eur. J. Neurosci.* **4**:46–60.

Del Río, M.R., and J. DeFelipe. 1996. Co-localization of calbindin D-28k, calretinin, and GABA immunoreactivities in neurons of the human temporal cortex. *J. Comp. Neurol.* **369**:472–482.

Del Rio, M.R., and DeFelipe J. 1997a. Co-localization of parvalbumin and calbindin D-28k in neurons including chandelier cells of the human temporal neocortex. *J. Chem. Neuroanat.* **12**:165–173.

Del Río, M.R., and J. DeFelipe. 1997b. Double bouquet cell axons in the human temporal neocortex: Relationship to bundles of myelinated axons and co-localization of calretinin and calbindin D-28k immunoreactivities. *J. Chem. Neuroanat.* **13**:243–251.

Demeulemeester, H., F. Vandesande, G.A. Orban, C. Brandon, and J.J. Vanderhaeghen. 1988. Heterogeneity of GABAergic cells in cat visual cortex. *J. Neurosci.* **8**:988–1000.

Demeulemeester, H., F. Vandesande, G.A. Orban, C.W. Heizmann, and R. Pochet. 1989. Calbindin D-28K and parvalbumin immunoreactivity is confined to two separate neuronal subpopulations in the cat visual cortex, whereas partial coexistence is shown in the dorsal lateral geniculate nucleus. *Neurosci. Lett.* **99**:6–11

Demeulemeester, H., L. Arckens, F. Vandesande et al. 1991. Calcium-binding proteins and neuropeptides as molecular markers of GABAergic interneurons in the cat visual cortex. *Exp. Brain Res.* **84**:538–544.

Dun, N.J., R. Huang, S.L. Dun, and U. Förstermann. 1994. Infrequent co-localization of nitric oxide synthase and calcium binding proteins immunoreactivity in rat neocortical neurons. *Brain Res.* **666**:289–294.

Eckenstein, F., and R.W. Baughman. 1984. Two types of cholinergic innervation in cortex, one co-localized with vasoactive intestinal polypeptide. *Nature* **309**:153–155.

Fairén, A., J. DeFelipe, and J. Regidor. 1984. Non-pyramidal neurons. General account. In: Cerebral Cortex, vol. 1, ed. A. Peters and E.G. Jones, pp. 201–253. New York: Plenum.

Ferezou, I., B. Cauli, E.L. Hill et al. 2002. 5-HT3 receptors mediate serotonergic fast synaptic excitation of neocortical vasoactive intestinal peptide/cholecystokinin interneurons. *J. Neurosci.* **22**:7389–7397.

Fuchs, E.C., H. Doheny, H. Faulkner et al. 2001. Genetically altered AMPA-type glutamate receptor kinetics in interneurons disrupt long-range synchrony of gamma oscillation. *PNAS* **98**:3571–3576.

Fukuda, T., and T. Kosaka. 2003. Ultrastructural study of gap junctions between dendrites of parvalbumin-containing GABAergic neurons in various neocortical areas of the adult rat. *Neuroscience* **120**:5–20

Gabbott, P.L.A., and S.J. Bacon. 1996. Local circuit neurones in the medial prefrontal cortex (areas 24 a,b,c, 25 and 32) in the monkey: II. Quantitative area l and laminar distributions. *J. Comp. Neurol.* **364**:609–636.

Gabbott, P.L.A., and S.J. Bacon. 1997. Vasoactive intestinal polypeptide containing neurones in monkey medial prefrontal cortex (mPFC): co-localisation with calretinin. *Brain Res.* **744**:179–184.

Galarreta, M., and S. Hestrin. 1999. A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature* **402**:72–75.

Geiger, J.R., T. Melcher, D.S. Koh et al. 1995. Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron* **15**:193–204.

Gibson, J.R., M. Beierlein, and B.W. Connors. 1999. Two networks of electrically coupled inhibitory neurons in neocortex. *Nature* **402**:75–79.

Goldberg, J.H., R. Yuste, and G. Tamas. 2003. Ca^{2+} imaging of mouse neocortical interneurone dendrites: Contribution of Ca^{2+}-permeable AMPA and NMDA receptors to subthreshold Ca^{2+}dynamics. *J. Physiol.* **551**:67–78.

Gonchar, Y., and A. Burkhalter. 1997. Three distinct families of GABAergic neurons in rat visual cortex. *Cereb. Cortex* **7**:347–358.

González-Albo, M.C., G.N. Elston, and J. DeFelipe. 2001. The human temporal cortex: Characterization of neurons expressing nitric oxide synthase, neuropeptides, calcium-binding proteins, and their glutamate receptor subunit profiles. *Cereb. Cortex* **11**:1170–1181.

Gupta, A., Y. Wang, and H. Markram. 2000. Organizing principles for a diversity of GABAergic interneurones and synapses in the neocortex. *Science* **287**:273–278.

Hendry S.H.C., and E.G. Jones. 1991. GABA neuronal subpopulations in cat primary auditory cortex: Co-localization with calcium binding proteins. *Brain Res.* **543**:45–55.

Hendry, S.H.C., E.G. Jones, J. DeFelipe et al. 1984a. Neuropeptide-containing neurones of the cerebral cortex are also GABAergic. *PNAS* **81**:6526–6530.

Hendry, S.H.C., E.G. Jones, and P.C. Emson. 1984b. Morphology, distribution and synaptic relations of somatostatin and neuropeptide Y-immunoreactive neurons in rat and monkey cortex. *J. Neurosci*. **4**:2497–2517.

Hendry S.H.C., E.G. Jones, P.C. Emson et al. 1989. Two classes of cortical GABA neurones defined by differential calcium binding protein immunoreactivities. *Exp. Brain Res.* **76**:467–472.

Hendry, S.H.C., H.D. Schwark, E.G. Jones, and J. Yan. 1987. Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *J. Neurosci.* **7**:1503–1519.

Hestrin, S. 1993. Different glutamate receptor channels mediate fast excitatory synaptic currents in inhibitory and excitatory cortical neurons. *Neuron* **11**:1083–1091.

Hormuzdi, S.G., I. Pais, F.E. LeBeau et al. 2001. Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice. *Neuron* **31**:487–495.

Joho, R.H., C.S. Ho, and G.A. Marks. 1999. Increased gamma- and decreased delta-oscillations in a mouse deficient for a potassium channel expressed in fast-spiking interneurons. *J. Neurophysiol.* **82**:1855–1864.

Jonas, P., C. Racca, B. Sakmann, P.H. Seeburg, and H. Monyer. 1994. Differences in Ca^{2+} permeability of AMPA-type glutamate receptor channels in neocortical neurons caused by differential GluR-B subunit expression. *Neuron* **12**:1281–1289.

Jones, E.G. 1975. Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. *J. Comp. Neurol.* **160**:205–268.

Jones, E.G., and S.H.C. Hendry. 1986. Co-localization of GABA and neuropeptides in neocortical neurons. *TINS* **9**:71–76.

Kanai, Y., and N. Hirokawa. 1995. Sorting mechanisms of tau and MAP2 in neurons: Suppressed axonal transit of MAP2 and locally regulated microtubule binding. *Neuron* **14**:421–432.

Kawaguchi, Y., and S. Kondo. 2002. Parvalbumin, somatostatin, and cholecystokinin as chemical markers for specific GABAergic interneuron types in the rat frontal cortex. *J. Neurocytol.* **31**:277–287.

Kawaguchi, Y., and Y. Kubota. 1993. Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindin D28k-immunoreactive neurons in layer V of rat frontal cortex. *J. Neurophysiol.* **70**:387–396.

Kawaguchi, Y., and Y. Kubota. 1997. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cereb. Cortex* **7**:476–486.

Keinanen, K., W. Wisden, B. Sommer et al. 1990. A family of AMPA-selective glutamate receptors. *Science* **249**:556–560.

Kisvárday, Z.F. 1992. GABAergic networks of basket cells in the visual cortex. *Prog. Brain Res.* **90**:385–405.

Kobayashi, K., P.C. Emson, C.Q. Mountjoy et al. 1990. Cerebral cortical calbindin D28K and parvalbumin neurones in Down's syndrome. *Neurosci. Lett.* **113**:17–22.

Kosaka, T., C.W. Heizmann, K. Tateishi, Y. Hamaoka, and K. Hama. 1987. An aspect of the organizational principle of the γ-aminobutyric acidergic system in the cerebral cortex. *Brain Res.* **409**:403–408.

Kosaka, T., M. Tauchi, and J.L. Dahl. 1988. Cholinergic neurons containing GABA-like and/or glutamic acid decarboxylase-like immunoreactivities in various brain regions of the rat. *Exp. Brain Res.* **70**:605–617.

Kowall, N.W., and M.F. Beal. 1988. Cortical somatostatin, neuropeptide Y, and NADPH diaphorase neurons: normal anatomy and alterations in Alzheimer's disease. *Ann. Neurol.* **23**:105–114.

Kubota, Y., R. Hattori, and Y. Yui. 1994. Three distinct subpopulations of GABAergic neurons in rat frontal agranular cortex. *Brain Res.* **649**:159–173.

Kubota, Y., and E.G. Jones. 1993. Co-localization of two calcium binding proteins in GABA cells of rat piriform cortex. *Brain Res.* **600**:339–344.

Kubota, Y., and Y. Kawaguchi. 1997. Two distinct subgroups of cholecystokinin-immunoreactive cortical interneurons. *Brain Res.* **752**:175–183.

Lambolez, B., N. Ropert, D. Perrais, J. Rossier, and S. Hestrin. 1996. Correlation between kinetics and RNA splicing of alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptors in neocortical neurons. *PNAS* **93**:1797–1802.

Laurie, D.J., P.C. Schrotz, H. Monyer, and U. Amtmann. 2002. Processing rodent embryonic and early postnatal tissue for *in situ* hybridization with radiolabelled oligonucleotides. *Intl. Rev. Neurobiol.* **47**:71–83.

Le Moine, C., and P. Gaspar. 1998. Subpopulations of cortical GABAergic interneurons differ by their expression of D_1 and D_2 dopamine receptor subtypes. *Brain Res. Mol. Brain Res.* **58**:231–236.

Leranth, C., Z. Szeidemann, M. Hsu, and G. Buzsaki. 1996. AMPA receptors in the rat and primate hippocampus: A possible absence of GluR2/3 subunits in most interneurons. *Neuroscience* **70**:631–652.

Leuba, G., and K. Saini. 1997. Co-localization of parvalbumin, calretinin, and calbindin D-28k in human cortical and subcortical visual structures. *J. Chem. Neuroanat.* **13**: 41–52.

Levitan, E.S., P.R. Schofield, D.R. Burt et al. 1988. Structural and functional basis for GABAA receptor heterogeneity. *Nature* **335**:76–79.

Lin, C., S.M. Lu, and D.E. Schmechel. 1986. Glutamic acid descarboxilase and somatostatin immunoreativities in rat visual cortex. *J. Comp. Neurol.* **244**:369–383.

Lorente de Nó, R. 1922. La corteza cerebral del ratón. (Primera contribución—La corteza acústica). *Trab. Lab. Invest. Biol. Madrid* **20**:41–78.

Lund, J.S. 1990. Excitatory and inhibitory circuiting and laminar mapping strategies in the primary visual cortex of the monkey. In: Signal and Sense: Local and Global Order in Perceptual Maps, ed. G.M. Edelman, W.E. Gall, and W.M. Cowan, pp. 51–82. New York: Wiley-Liss.

Margrie, T.W., A.H. Meyer, A. Caputi et al. 2003. Targeted whole-cell recordings in the mammalian brain *in vivo*. *Neuron* **39**:911–918.

Marin-Padilla, M. 1969. Origin of the pericellular baskets of the pyramidal cells of the human motor cortex: A Golgi study. *Brain Res.* **14**:633–646.

Marsicano, G., and B. Lutz. 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* **11**:4213–4225.

Matsuda, L.A., T.I. Bonner, and S.J. Lolait. 1993. Localization of cannabinoid receptor mRNA in rat brain. *J. Comp. Neurol*. **327**:535–550.

McBain, C.J., and A. Fisahn. 2001. Interneurons unbound. *Nat. Rev. Neurosci*. **2**:11–23.

Meskenaite, V. 1997. Calretinin-immunoreactive local circuit neurons in the area 17 of the cynomolgus monkey, Macaca fascicularis. *J. Comp. Neurol*. **379**:113–132.

Meyer, A.H., I. Katona, M. Blatow, A. Rozov, and H. Monyer. 2002. In vivo labeling of parvalbumin-positive interneurons and analysis of electrical coupling in identified neurons. *J. Neurosci*. **22**:7055–7064.

Monyer, H., N. Burnashev, D.J. Laurie, B. Sakmann, and P.H. Seeburg. 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**:529–540.

Monyer, H., and H. Markram. 2004. Molecular and genetic tools to study GABAergic interneurone diversity and function. *TINS* **27**:90–97.

Monyer, H., P.H. Seeburg, and W. Wisden. 1991. Glutamate-operated channels: Developmentally early and mature forms arise by alternative splicing. *Neuron* **6**:799–810.

Morales, M., and F.E. Bloom. 1997. The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J. Neurosci*. **17**:3157–3167.

Morales, M., E. Battenberg, L. de Lecea, and F.E. Bloom. 1996. The type 3 serotonin receptor is expressed in a subpopulation of GABAergic neurons in the rat neocortex and hippocampus. *Brain Res*. **731**:199–202.

Papadopoulos, G.C., J.G. Parnavelas, and M.E. Cavanagh. 1987. Extensive co-existence of neuropeptides in the rat visual cortex. *Brain Res*. **420**:95–99.

Porter, J.T., B. Cauli, K. Tsuzuki et al. 1999. Selective excitation of subtypes of neocortical interneurons by nicotinic receptors. *J. Neurosci*. **19**:5228–5235.

Ren, J.Q., Y. Aika, C.W. Heizmann, and T. Kosaka. 1992. Quantitative analysis of neurons and glial cells in the rat somatosensory cortex, with special reference to GABAergic neurons and parvalbumin-containing neurons. *Exp. Brain Res.* **92**:1–14.

Roerig, B., D.A. Nelson, and L.C. Katz. 1997. Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT3 receptors in developing visual cortex. *J. Neurosci.* **17**:8353–8362.

Rogers, J.H. 1992. Immunohistochemical markers in rat cortex: Co-localization of calretinin and calbindin-D28k with neuropeptides and GABA. *Brain Res.* **587**: 147–157.

Rogers, J.H., and A. Resibois 1992. Calretinin and calbindin-D28k in rat brain: Patterns of partial co-localization. *Neuroscience* **51**:843–865.

Rozov, A., J. Jerecic, B. Sakmann, and N. Burnashev. 2001. AMPA receptor channels with long-lasting desensitization in bipolar interneurons contribute to synaptic depression in a novel feedback circuit in layer 2/3 of rat neocortex. *J. Neurosci.* **21**:8062–8071.

Schwark, H.D., and J. Li. 2000. Distribution of neurons immunoreactive for calcium-binding proteins varies across areas of cat primary somatosensory cortex. *Brain Res. Bull.* **51**:379–385.

Seeburg, P.H. 1993. The TINS/TiPS Lecture: The molecular biology of mammalian glutamate receptor channels. *TINS* **16**:359–365.

Silberberg, G., A. Gupta, and H. Markram. 2002. Stereotypy in neocortical microcircuits. *TINS* **25** 227–230.

Smiley, J.F., J.P. McGinnis, and D.C. Javitt. 2000. Nitric oxide synthase interneurons in the monkey cerebral cortex are subsets of the somatostatin, neuropeptide Y, and calbindin cells. *Brain Res.* **863**:205–212.

Somogyi, P., A.J. Hodgson, A.D. Smith et al. 1984. Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. *J. Neurosci.* **4**:2590–2603.

Somogyi, P., G. Tamás, R. Lujan, and E.H. Buhl. 1998. Salient features of synaptic organisation in the cerebral cortex. *Brain Res. Rev.* **26**:113–135.

Standaert, D.G., G.B. Landwehrmeyer, J.A. Kerner, J.B. Penney, Jr., and A.B. Young. 1996. Expression of NMDAR2D glutamate receptor subunit mRNA in neurochemically identified interneurons in the rat neostriatum, neocortex, and hippocampus. *Mol. Brain Res.***42**:89–102.

Stinehelfer, S., M. Vruwink, and A. Burette. 2000. Immunolocalization of mGluR1alpha in specific populations of local circuit neurons in the cerebral cortex. *Brain Res.* **861**:37–44.

Taki, K., T. Kaneko, and N. Mizuno. 2000. A group of cortical interneurons expressing mu-opioid receptor-like immunoreactivity: A double immunofluorescence study in the rat cerebral cortex. *Neuroscience* **98**:221–231.

Tamamaki, N., Y. Yanagawa, R. Tomioka et al. 2003. Green fluorescent protein expression and co-localization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J. Comp. Neurol.* **467**:60–79, doi: 10.1002/cne.10905.

Tamás, G., E.H. Buhl, A. Lorincz, and P. Somogyi. 2000. Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nat. Neurosci.* **3**:366–371.

Tamás, G., E.H. Buhl, and P. Somogyi. 1997. Massive autaptic self-innervation of GABAergic neurons in cat visual cortex. *J. Neurosci.* **17**:6352–6364.

Thomson, A.M., and J. Deuchars. 1997. Synaptic interactions in neocortical local circuits: Dual intracellular recordings *in vitro*. *Cereb. Cortex* **7**:510–522.

Trottier, S., M. Geffard, and B. Evrard. 1989. Co-localization of tyrosine hydroxylase and GABA immunoreactivities in human cortical neurons. *Neurosci. Lett.* **106**:76–82.

Tsou, K., S. Brown, M.C. Sanudo-Pena, K. Mackie, and J.M. Walker. 1998. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**:393–411.

van Brederode, J.F.M., K.A. Mulligan, and A.E. Hendrickson. 1990. Calcium-binding proteins as markers for subpopulations of GABAergic neurons in monkey striate cortex. *J. Comp. Neurol.* **298**:1–22.

Venance, L., A. Rozov, M. Blatow et al. 2000. Connexin expression in electrically coupled postnatal rat brain neurons. *PNAS* **97**:10,260–10,265.

Vickers, J.C., G.W. Huntley, A.M. Edwards et al. 1993. Quantitative localization of AMPA/kainate and kainate glutamate receptor subunit immunoreactivity in neurochemically identified subpopulations of neurons in the prefrontal cortex of the macaque monkey. *J. Neurosci*. **13**:2982–2992.

Waldvogel, H.J., Y. Kubota, J. Fritschy, H. Mohler, and R.L. Faull. 1999. Regional and cellular localisation of GABA(A) receptor subunits in the human basal ganglia: An autoradiographic and immunohistochemical study. *J. Comp. Neurol.* **415**:313–340.

Weiser, M., E. Vega-Saenz de Miera, C. Kentros et al. 1994. Differential expression of Shaw-related K+ channels in the rat central nervous system. *J. Neurosci*. **14**:949–972.

Wenthold, R.J., N. Yokotani, K. Doi, and K. Wada. 1992. Immunochemical characterization of the non-NMDA glutamate receptor using subunit-specific antibodies. Evidence for a hetero-oligomeric structure in rat brain. *J. Biol. Chem.* **267**:501–507.

White, E.L. 1989. Cortical Circuits: Synaptic Organization of the Cerebral Cortex. Structure, Function and Theory. Boston: Birkhäuser.

Whittington, M.A., R.D. Traub, and J.G. Jefferys. 1995. Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature* **373**:612–615.

16

UP States and Cortical Dynamics

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ABSTRACT

Neuronal networks within the cerebral cortex are highly recurrent, both locally and globally, with each cell typically projecting to and receiving inputs from thousands of other neurons. This pattern of highly divergent and convergent recurrent connectivity leads to a strong automodulatory influence of the cortex upon itself. As predicted by theorists, self-regulation could allow the cortex to exhibit autonomous dynamical behavior and to enter into behaviorally relevant and useful states. Using *in vitro* slice preparations, we demonstrate that local cortical networks produce rapid (<100 ms) transitions between relatively stable UP (depolarized) or DOWN (resting) states. Two types of UP states synchronizations have been described: global UP states, which involve most neurons, and local UP states, produced by a small percentage of the cells. The relation between these two types of UP states is still poorly understood. UP states are maintained through balanced recurrent activity and may last for several seconds. Activation of inputs can both start and stop this persistent recurrent discharge, and UP states could be associated with a marked increase in responsiveness of cortical neurons to small synaptic inputs. We propose that the temporary formation of coactive neuronal assemblies is a fundamental operating feature of the cerebral cortex. This functional property of cortical networks could implement circuit attractors and mediate working memory, attention, sensorimotor coordination, and other computational functions of cortical circuits.

INTRODUCTION

Since the time of Cajal, and later Lorente de Nó and Hebb, a long-standing theory of brain function holds that the nervous system operates through the temporary formation of neuronal ensembles—networks of coactive and synaptically interacting neurons. The dynamic activation of neuronal assemblies in the cerebral cortex has been proposed to underlie a diverse range of cognitive functions

including working memory, attention, coupling of different sensory inputs to varying motor responses, and even thought itself. The central tenet of these theories is that the cortex is a highly dynamic structure, able to form assemblies of coactive neurons on the fly—and these temporary junctions of neuronal interaction allows the cortex to bring together quickly a wide variety of sensory/motor/cognitive processes for a behavioral task and to disassemble them just as quickly when they are no longer needed (e.g., using the same motor program to pick up a cup located in different parts of the visual field requires the dynamic coupling of different sensory inputs to the same motor output). The direct study of cortical neuronal assemblies *in vivo* has been difficult, owing to the very large number of neurons in the neocortex, and our present inability to record directly from more than a handful of them simultaneously. Recently, *in vitro* cortical slice preparations have revealed the generation of repeating periods of neuronal activity through the recurrent activation of neuronal assemblies (Sanchez-Vives and McCormick 2000; Mao et al. 2001; Cossart et al. 2003; Shu et al. 2003a, b; Seamans et al. 2003). Slice preparations have been useful in gaining insight into the mechanisms by which recurrent cortical network activity is generated and how it may control neuronal responsiveness, and thus, the flow of information within the cerebral cortex. Thus, the study of functional properties of cortical circuits *in vitro* is providing information about how these may contribute to global brain function *in vivo*. The aim and scope of our chapter is to explain local circuit dynamics in the cerebral cortex and how these may contribute to cortical processing.

CORTICAL NETWORKS GENERATE UP AND DOWN STATES *IN VIVO* AND *IN VITRO*

In both neocortex and striatum, intracellular recordings in anesthetized animals often reveal the recurring transition between two semistable states: a period of quiescence in the cortical network (so-called DOWN state) followed by a period of low-firing rate (e.g., 1–20 Hz) activity (UP state; see Figure 16.1) (Wilson and Groves 1981; Stériade et al. 1993, 2001; Cowan and Wilson 1994). This alternation between UP and DOWN states is also found in naturally sleeping animals, occurs in all cells (both pyramidal and nonpyramidal) recorded, and recurs at frequencies of between 0.1 and 1 Hz (Stériade et al. 2001). *In vivo*, the UP state lasts from 0.1 to several seconds, while the DOWN state is typically 0.1 to 0.5 seconds long (Stériade et al. 2001). Surprisingly, a very similar pattern of activity is also found in adult ferret and mouse neocortical slices (Figure 16.1; Sanchez-Vives and McCormick 2000; Shu et al. 2003a, b; Cossart et al. 2003), organotypic cortical slice cultures (Seamans et al. 2003; Aptowicz and McCormick, unpublished observations), and even in networks of dissociated cultured neocortical neurons (Nemet and Yuste, unpublished observations).

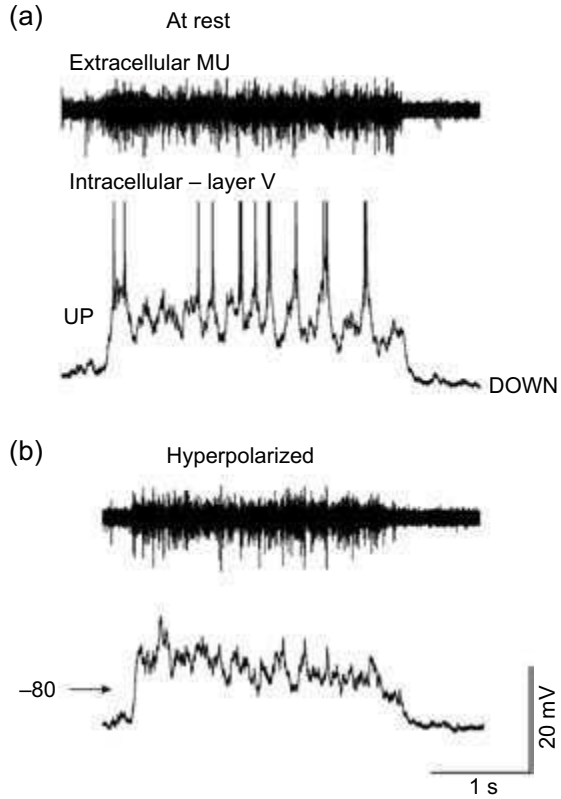

Figure 16.1 The ferret prefrontal cortical slice in the interface chamber *in vitro* spontaneously generates prolonged periods of activity through synaptic bombardments. (a) Simultaneous intracellular and extracellular recordings in layer V of the ferret prefrontal cortex. The network enters into the UP state for approximately 3 seconds prior to a rapid transition to the DOWN state. (b) Hyperpolarization of the intracellularly recorded pyramidal cell reveals the barrage of postsynaptic potentials arriving during the UP state and the reduction of postsynaptic potentials during the DOWN state. Note that hyperpolarization or depolarization does not affect the duration of the UP state, which is the same as the duration of action potential discharge in the local network. MU: multiple unit activity. From McCormick et al. (2003).

Global UP States in Ferret Slices and in Anesthetized Preparations *in Vivo*

Two types of UP/DOWN states have been reported. The sleep/anesthesia/ferret *in vitro* UP/DOWN states are "global"; that is, all or nearly all cortical neurons are simultaneously involved, discharging during the UP and being relatively silent during the DOWN periods. Interestingly, this activity shows rapid transitions between states—within approximately 100 ms or so (Figure 16.1). This feature may allow the activation of recurrent networks to control neuronal

responsiveness rapidly (see below). In cortical slices, the transitions between states propagates laterally at about 11 mm/s, while *in vivo* these transitions exhibit phase delays that are consistent with rapid propagation (>100 mm/s) that probably occurs in a complex fashion, traveling in both anterior–posterior and medial–lateral directions, as well as skipping from area to area in accordance with the density of functional connectivity and refractoriness of each region of the network. These results indicate that a fundamental feature of local cortical networks throughout the cerebrum is the ability to generate different stable states of activity through recurrent excitation and inhibition.

What Normally Starts and Stops Global UP States?

If transitions between stable states is a fundamental feature of cortical networks that is used, in some form (perhaps as transitions of smaller amplitude), to control neuronal responsiveness or to act as temporary "memories," then these stable patterns of activity should be controlled by afferent inputs. Indeed, electrical stimulation of afferent pathways in the cortical slice can cause the rapid transition between states—from UP to DOWN and from DOWN to UP (Shu et al. 2003a). Intracellular recordings from pyramidal and fast-spiking inhibitory interneurons during these transitions reveal a possible mechanism. Delivery of an extracellular stimulus while the network is in the DOWN state results in the excitation of pyramidal cells as well as fast-spiking interneurons, and the arrival of EPSP barrages in both cell types. If the stimulus is moderate in amplitude (so as not to activate strongly inhibitory interneurons), then the excitation is allowed to reverberate within the network, causing persistent activity. If the afferent pathway (or another afferent) is subsequently activated during the UP state, it often terminates the persistent activity, causing a transition to the DOWN state. How can afferent inputs terminate the UP state? Intracellular recordings from fast-spiking cells demonstrate that the activation of an afferent input during the UP state causes these inhibitory cells to generate a high-frequency burst of action potentials. This burst of action potentials results from the prolonged barrage of EPSPs that fast-spiking neurons receive in response to afferent inputs, as well as the nearness of the cell to firing threshold during the UP state. The prolonged discharge of fast-spiking GABAergic neurons results in excess inhibition in the network, and it is likely that this contributes to the cessation of the persistent activity of the UP state (Shu et al. 2003a). These results demonstrate that the activation of afferents may control the timing of transitions between various states of excitability in cortical networks. In addition, the UP and DOWN state transitions may occur spontaneously. The transition from DOWN to UP may occur when a critical number of excitatory neurons spontaneously discharge near the end of the refractory period. The opposite transition, from UP to DOWN, may occur as the result of buildup of adaptive mechanisms, such as spike frequency adaptation or synaptic depression (Compte et al. 2003).

Local UP States in Mouse Cortical Slices

In contrast to this high percentage of participation in network UP states *in vivo* and ferret slices *in vitro*, mouse visual cortical slices (P14–P21) spontaneously generate sparse synchronized activity in which few neurons simultaneously enter UP states (Figure 16.2; Cossart et al. 2003). These "local" UP states were discovered by imaging spontaneous calcium dynamics in large populations of cortical neurons. Since action potentials faithfully generate large somatic calcium transients, calcium imaging can be used as an indirect method to monitor the spiking activity of a large neuronal population.

In this two-photon imaging study, small groups of spontaneously coactive cells appeared in different positions of the slice, forming in some cases striking spatial patterns. These spatial patterns were sometimes consistent with known anatomical features of the circuit such as layers, sublayers, or vertical columns, although most patterns were dispersed (Figure 16.2). Moreover, whole-cell recordings of neurons that participated in this synchronization demonstrated the existence of spontaneous UP states, whose associated action potentials indeed generated the calcium transients (Figure 16.3). Thus, this imaging method

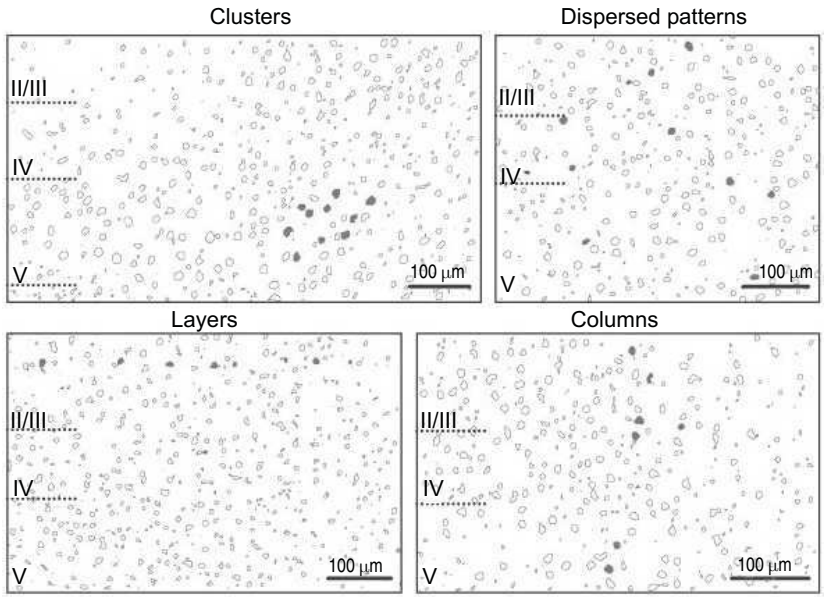

Figure 16.2 Spatial structure of synchronous neuronal ensembles (local UP states) in slices of mouse visual cortex. Representative contourplots of four types of spatial arrangement of cells coactive during spontaneous synchronizations. Black contours indicate cells active at peak of synchrony. Reprinted with permission from Cossart et al. (2003).

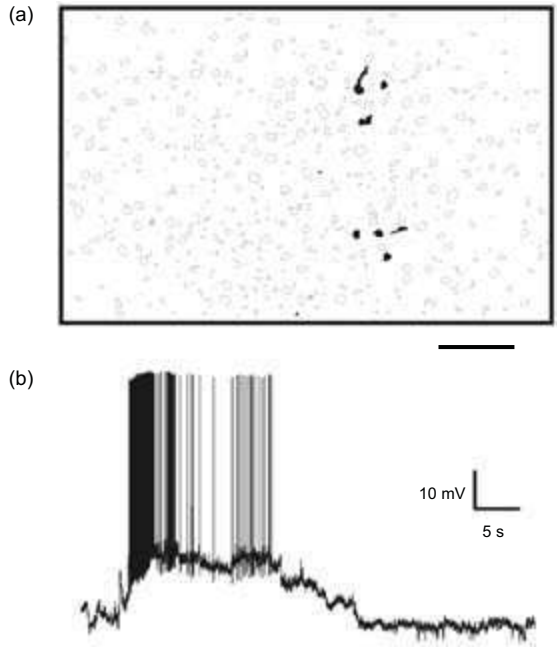

Figure 16.3 Synchronous activity in slices is mediated by UP states. (a) Simultaneous imaging of network activity and current-clamp recording of a pyramidal cell active at multiple synchronizations. Black contours indicate active cells. Optical signals corresponded to shifts of the membrane potential to an UP state. Scale bar: 100 μm. (b) Membrane potential distribution of a representative example. Reprinted with permission from Cossart et al. (2003).

enables the simultaneous detection of UP states in populations of up to 4000 neurons.

Local UP states have intracellular properties that are similar to those global UP states previously described in other preparations. Specifically, at the single-cell level, local UP states maintained the membrane potential approximately 10 mV depolarized from rest and generated barrages of action potentials at ~20 Hz. UP states turned on in ~60 ms, lasted for hundreds of ms, and turned off in ~160 ms. Some cells generated UP states that persisted for several seconds.

The temporal dynamics of the network synchronization generated by these UP states appeared to be stereotyped (Cossart et al. 2003) (Figure 16.4). Synchronization among neurons entering UP states was slow and could take several seconds to complete. The same groups of neurons could become synchronized more than once, yet the spatiotemporal patterns of these repeated synchronizations were always somewhat different from case to case. Finally, network synchronizations could be triggered by extracellular electrical stimulation (Cossart, Aronov, and Yuste, unpublished).

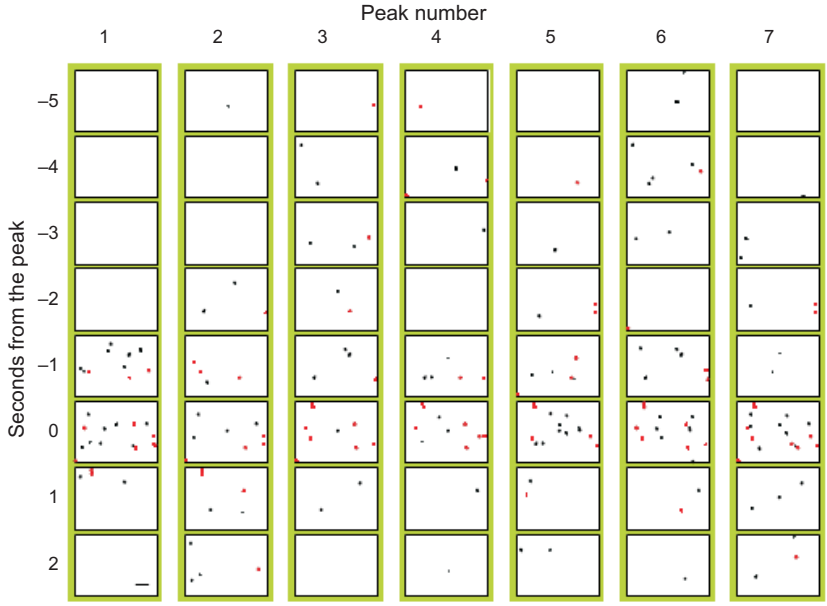

Figure 16.4 Stereotypical spatiotemporal dynamics of local UP states. Sequences of consecutive movie frames before and after seven different repeated peaks of synchrony. Only active cells are indicated. The cells active at every synchronization are indicated in blue. Red contours indicate active cells. All imaged cells are shown at frame 5. Scale bar: 100 μm. Reprinted with permission from Cossart et al. (2003).

Although local UP states share many similarities with global UP states, they differ in their spatial extent. The reasons underlying these differences are not yet understood. Explanations could be the differences in species (mouse vs. ferret), or age (juvenile vs. adult tissue). Also, the presence of anesthetics or changes in divalent concentrations could generalize local UP states. Finally, the submerged versus interface slice preparations could also contribute to these differences. The relation between either type of UP state and the physiology of the awake, behaving animal is as yet unknown.

Attractor Dynamics of Local UP States

The stereotypical dynamics of network synchronizations based on local UP states are reminiscent of attractor dynamics, emergent properties of artificial neural networks (Hopfield 1982). In these feedback circuits, the recurrent excitatory connectivity dominates and can produce stable states in the dynamics, which can be visualized as valleys in an energy landscape of the population dynamics. Because the UP states can outlast the (assumed) fast duty cycle of the

cortical circuit, they could be implementing circuit attractors. Attactors could represent the solution to a specific computation (Hopfield 1982), and have been used extensively to model orientation selectivity eye position stability and working memory (Wang 2001). Attractors are also the preferred theoretical models in the analysis of biochemical and genetic networks and are a good example of an emergent property of systems, one, which does not exist unless the interactions between individual elements are taken into account.

Dense versus Distributed UP State Participation in Neuronal Assemblies

The UP and DOWN states generated *in vivo* during sleep and anesthesia as well as in ferret cortical slices *in vitro* are characterized by a dense, high-percentage participation of neighboring neurons. As explained, in cortical slices from mice, in contrast, the participation is much more sparse (Cossart et al. 2003). If the persistent activity we are investigating *in vitro* is to be used as a model of persistent activity in cortical areas during working memory (Goldman-Rakic 1995; Fuster 1995), then a question arises as to the degree of local participation of neurons in this activity in awake, behaving animals. Neighboring cells recorded on the same microelectrode in the prefrontal cortex, for example, can have very different activities with only one of the cells discharging during the delay (mnemonic) period. This suggests that during working memory tasks, there is a locally sparse participation of neurons in the delay period activity. However, simultaneous recordings of multiple and single unit activity recorded from the same electrode typically show the same pattern of activity in both types of recording (e.g., same "memory fields"), indicating that the local representation is not *very* sparse (Constantinidis et al. 2001). As yet, it is unclear how cortical persistent activity may be generated with both sparse and dense local representation — and especially how sparse local activity is controlled so as to prevent it from becoming a dense local representation. It should be emphasized that a locally sparse representation does not necessarily imply that very few neurons participate in the persistent activity — the active cells may be distributed over distal and/or multiple cortical regions. One hypothesis is that the transition from a locally sparse to a locally dense participation depends upon some critical number of local neurons to enter into the UP state. Once this threshold is passed, then the activity rapidly propagates through the local network. Perhaps *in vivo* in an UP state with locally sparse activity, the participation of neurons that are distal provide sufficient depolarization to maintain a fraction of local cells in the persistent activity state, but not enough general activation of the local cortical area to cause all of the neurons to transit into the UP state. Evidence for this hypothesis is the rapid rise and fall of UP states *in vitro*: when a critical number of cells stop discharging from refractory mechanisms, the entire network fails and the recurrent activity quickly turns off.

Persistent Activity Can be Generated Through a Precise Balance of Excitation and Inhibition

The two main categories of cortical neuron are the excitatory (pyramidal, spiny stellate) and inhibitory (GABAergic) neurons. Both of these cell types probably have dozens of morphological and physiological subtypes, each of which could implement different circuit functions. A basic feature of interconnectivity of the cortex is that excitatory cells generate recurrent feedback and feedforward excitation to one another in an anatomically precise manner, at least in some cases (Silberberg et al. 2002). This recurrent connectivity leads to the presence of strong positive feedback loops within cortical networks. Local inhibitory neurons may act to constrain, guide, and control this recurrent connectivity and are thought to project with a high degree of target precision. Interposed within each excitatory projection is an additional projection to GABAergic inhibitory neurons, which then feed a dampening influence into the local cortical network. In this manner, a precise proportionality between recurrent excitation and inhibition could be established. As recurrent excitation increases and decreases, so does recurrent inhibition, resulting in, effectively, a "reversal potential clamp" (Figure 16.5). Recent computational models of the cerebral cortex often assume such proportionality of recurrent excitation and inhibition, which has the distinct advantage of allowing the generation of periods of stable activity (see Wang 2001). Alternative models of generation of persistent activity have focused on the intrinsic properties of neurons (e.g., Egorov et al. 2002).

Recent intracellular recordings *in vivo* during the spontaneous generation of UP states in anesthetized animals also reveal a marked balance between recurrent excitation and inhibition (Haider, Duque, Hasenstaub, and McCormick, unpublished observations), even though there is a preservation of the massive recurrent connectivity of the cortex. This is as expected from the *in vitro* results, since recurrent excitation and inhibition are balanced, no matter what the value of the recurrent excitation (within reason), the inhibition will be adjusted (through greater firing of inhibitory neurons, for example) to match it.

Implications of Balanced Excitation and Inhibition in Cortical Networks

A strict balance or proportionality between excitation and inhibition in the cortex has multiple consequences on the manner in which cortical networks operate (Figure 16.6). First, strong recurrent inhibition could allow for the rapid and ongoing control of neuronal activities and responses in the cortex, preventing them from becoming more dominant than is behaviorally appropriate. An extreme example of this is epileptic seizures, in which recurrent excitatory networks are left relatively unchecked during a seizure. Milder forms of imbalance of recurrent excitation and inhibition may underlie a wide range of psychiatric disorders by allowing the generation of recurring inappropriate patterns of activity between

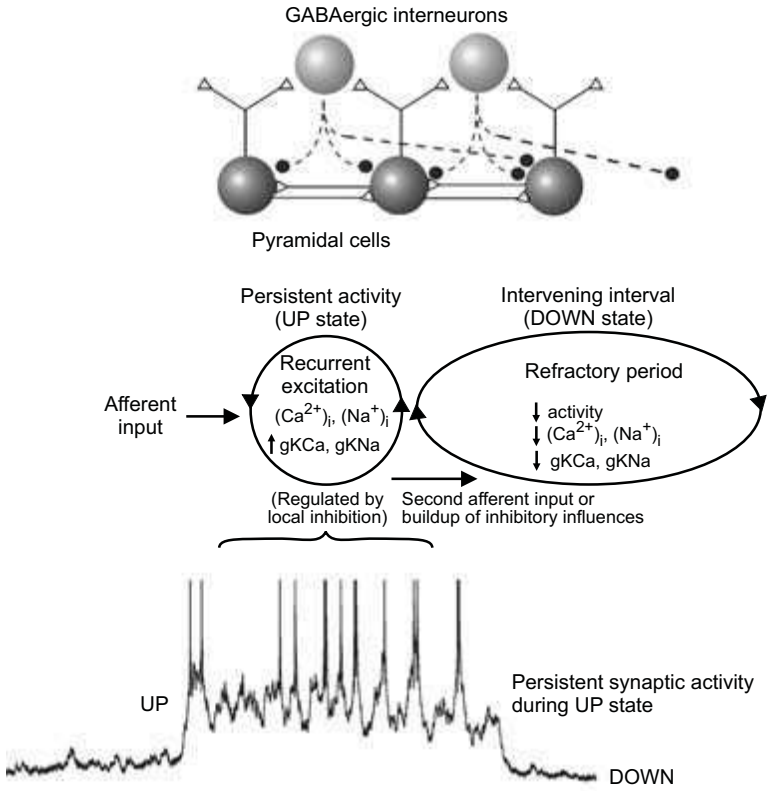

Figure 16.5 Summary diagram of the proposed mechanisms for the spontaneous generation of the UP and DOWN states in cortical networks. Cortical pyramidal and local GABAergic interneurons are highly interconnected through local axonal connections such that activity in pyramidal cells excites both other pyramidal cells as well as local interneurons. The inhibitory feedback from the local interneurons controls the level of the UP state. Activation of an afferent input can trigger the transition from the DOWN to the UP state. Buildup of intracellular levels of Ca^{2+} and Na^+ can activate K^+ currents, which eventually tip the balance back to the DOWN state, during which time the levels of Ca^{2+} and Na^+ inside the cell decrease, thus allowing the network to generate spontaneously another UP state. Another mechanism for making the transition from the UP to the DOWN state is the activation of afferent inputs. These mechanisms are based on the studies of global UP states and may not generalize to local UP states.

excitatory neurons. Examples include schizophrenic hallucinations, obsessive thoughts in obsessive compulsive disorder (OCD), and the tics of Tourette's syndrome. These disorders must reflect unusual and inappropriate discharge of neuronal assemblies in the cortex—and since the balance of excitation and inhibition regulates this, this balance is necessarily involved.

Another feature of cortical networks that may depend upon an ongoing balance of excitation and inhibition is the generation of action potentials in a

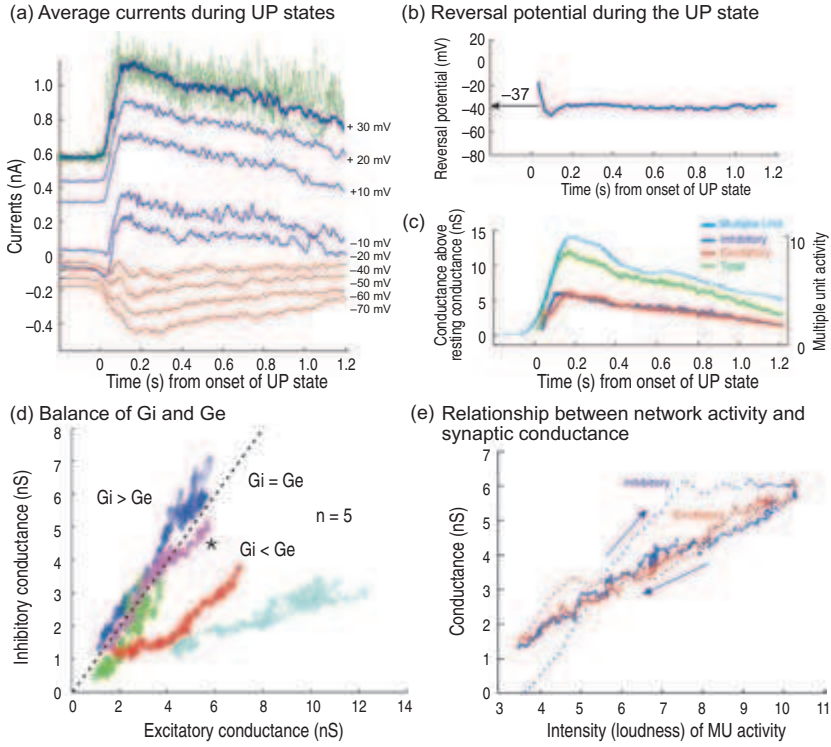

Figure 16.6 Recurrent activity is generated by a balanced barrage of inhibitory and excitatory postsynaptic potentials. (a) Average currents during the UP state under voltage clamp. Each trace is an average of 9–17 trials. Several raw traces at +30 mV are shown with the average for comparison. The average synaptic currents reverse around –37 mV in this cell. Electrode contained 2 M CsAc and 50 mM QX-314 to minimize the contribution from K^+ and Na^+ currents. Similar results were obtained with electrodes containing KAc only. (b) Calculation of the reversal potential of the average synaptic currents over time. (c) Illustration of the amplitude-time course during the UP state of the average multiple unit (MU) activity, the total increase in conductance (as measured by the change in slope of the IV plot), and the calculated conductance of excitatory and inhibitory currents. (d) Plot of the inhibitory conductance as a function of the excitatory conductance in five different neurons. (e) Relationship between the average intensity of neuronal activity in the local network, as measured by MU activity, and the amplitude of the calculated excitatory and inhibitory conductances. In this cell, as the network transitioned into the UP state, the inhibitory conductance lagged the excitatory conductance. Following the onset of the UP state, the excitatory and inhibitory conductances were proportional and strongly correlated with the intensity of activity in the nearby (<100 μm) layer V multiple unit recording. From Shu et al. (2003a).

stochastic manner (Shadlen and Newsome 1998). Since neuronal action potential generation is relatively accurate and repeatable, the trial-to-trial and within-trial variations in spike timing are believed to result from the nearly random

appearance of depolarizing synaptic events that cross spike threshold. A network of excitation and inhibition that is balanced such that the membrane potential is poised near firing threshold may generate this type of precise activity. Such a balanced network can respond to synchronized inputs in a rapid and accurate manner, since they are poised near firing threshold and the membrane time constant is relatively short, owing to ongoing activity (Salinas and Sejnowski 2001).

Do UP and DOWN States Occur in the Waking Animal?

The first description of spontaneous periods of persistent depolarized states was carried out in intracellular recordings of neostriatal neurons from paralyzed rats (Wilson and Groves 1981). In neocortical neurons, intracellular recordings from cats during the transition to the waking state reveal a cessation of frank UP and DOWN states and a maintained depolarization that is near firing threshold (Stériade et al. 2001). However, closer examination of these membrane potentials in the waking animal reveal deviations on the order of 5 mV, which is more than large enough to have the types of effects on neuronal responsiveness that are typically seen *in vivo* with alterations in attention, excitability, and such. In addition, variations in neuronal excitability and responsiveness, consistent with processes somewhat similar to UP and DOWN states, have been reported in recordings from awake and behaving monkeys (Super et al. 2003; Leopold et al. 2003). For example, variations in neuronal activity in V1 that preceded the task were highly correlated with the ability of the animal to detect a figure-ground segregation task (Super et al. 2003), even though the animal was awake and attentive during the entire period. Another example of possible UP- and DOWN-like activities in the visual system is that of binocular rivalry. The perceptual appearance of an input from one eye results in the suppression of that from the other, and this suppression can even propagate as a traveling wave (Wilson et al. 2001). Binocular rivalry is often modeled as a winner-take-all situation with suppression of competing activity patterns, and winner-take-all algorithms could be easily implemented by circuit attractors. We hypothesize that the winning network is those neurons that interactively enter into an UP-like state. Finally, Changeux and Michel (this volume) provide evidence for rapid transitions between microstates in the cerebral cortex and their possible relation to cognition.

Control of Neuronal Responsiveness through Recurrent Network Activity

The possibility that rapid barrages of synaptic activity may be used to control the flow of information within the cerebral cortex has led many investigators to examine the precise influence of this activity on neuronal responsiveness. Barrages of synaptic potentials arriving in cortical cells during the generation of UP

states result in changes in three main parameters: a depolarization of the membrane potential, an increase in membrane conductance, and an increase in membrane variance (often referred to as membrane "noise") (see Figure 16.1). How will each of these three components of synaptic barrages influence the response properties of cortical neurons? Theoretical and biological studies give a partial answer to this actively investigated question (Hô and Destexhe 2000; Chance et al. 2002; Shu et al. 2003b; Anderson et al. 2000). In the absence of membrane variance (e.g., noise), neurons act more or less as threshold detectors, discharging when an isolated EPSP is large enough to cross threshold (although the rate of depolarization to threshold and the spike history of the neuron also has an effect). Tonic depolarization of cortical cells merely results in a decrease in threshold, with smaller EPSPs reaching spike threshold, since there is a smaller difference between the resting membrane potential and spike threshold. However, tonic increases in membrane conductance result in the opposite, a larger amplitude EPSP is required to reach spike threshold, owing to the decreased ability of these to depolarize the neuron. More interestingly, the addition of membrane variance (noise), such as would occur during barrages of postsynaptic potentials, results in a shift in the firing mode of cortical cells from one of threshold detection to a probabilistic discharge, with the probability, over many trials, of discharge being dictated by the amplitude-time course of the nonnoisy input. In a network of cells, random noise may therefore paradoxically facilitate the encoding of a common signal through the variation of spike probabilities within each of the participating cells (Anderson et al. 2000). Combining the depolarization, increase in membrane conductance, and increase in membrane variance together, as occurs when cortical neurons are bombarded with depolarizing barrages of synaptic potentials, results in a marked increase in sensitivity to small inputs, an increase in correlation between input waveform and spike rate, and a change in slope of the input–output relation (Shu et al. 2003b). Indeed, *in vivo* studies suggest that the activation of the UP states results in a marked increase in neuronal responsiveness to synaptic or sensory inputs (Timofeev et al. 1996; Haider, Duque, and McCormick, unpublished observations).

POTENTIAL FUNCTION OF UP STATES: AROUSAL, ATTENTION, AND MEMORY

Our combined results reveal two different types of network UP states and indicate that a basic operation of the cerebral cortex is the generation of self-sustained periods of activity. Intracortically generated UP states have been conclusively observed so far only during slow wave sleep or anesthesia (Stériade et al. 1993, 2001; Anderson et al. 2000). However, the mechanisms by which they are generated has important implications for our understanding of the cellular basis not only of sleep rhythms, but also potentially of working memory, attention, and arousal.

Stimulation of ascending activating systems in the brainstem results in activation of the EEG (e.g., a pattern of EEG activity similar to waking), even in anesthetized animals. Intracellular recordings in cortical pyramidal cells during the slow oscillation reveal that brainstem stimulation results in an abolition of the DOWN state and a maintenance of the membrane potential at a depolarized level with a degree of noisiness that is similar to that of the UP state (Stériade et al. 2001). This result would suggest that the UP state is similar to the resting state of the waking brain. However, intracellular recordings *in vivo* during natural sleep and waking reveal that the input resistance of pyramidal cells in the waking state may be significantly higher than during the UP state and that *the membrane potential may still exhibit significant depolarizing and hyperpolarizing jumps in the waking state*. The mechanisms underlying the apparent increase in input resistance during the natural waking state in comparison to the UP state remain to be uncovered. This difference suggests that the UP state may be distinct from simply the resting state of the cell in the waking state. The UP state, therefore, has some, but not all, properties of several aspects of cortex function in the waking animal, including a maintained depolarization and persistent activity, an increase in neuronal excitability, and spontaneous low-frequency discharge.

Recordings in multiple regions of the brain reveal that working memory tasks (in which a feature of a stimulus must be remembered for several seconds) result in the persistent discharge at rates of 5–40 Hz of selected cortical (and subcortical) neurons during the "memory" or delay period (Goldman-Rakic 1995; Fuster 1995). This persistent activity has been hypothesized to be generated either through recurrent excitatory and inhibitory interactions (Goldman-Rakic 1995; Fuster 1995) or through the activation of intrinsic membrane mechanisms (Egorov et al. 2002), although these mechanisms are not mutually exclusive. Our results demonstrate that local recurrent networks within the cortex are perfectly capable of generating second-long periods of sustained activity mediated through the properties of recurrent excitation controlled by inhibition (Cossart et al. 2003; Shu et al. 2003a, b). We have not yet found a strong role for intrinsic membrane properties in the generation of the global UP period. For example, hyperpolarization of the recorded neuron does not result in an abolition of the UP state nor does it change its duration or rate of recurrence. In addition, neuronal discharge during the delay period exhibits a broad distribution of interspike intervals, which is not seen when persistent activity is generated through intrinsic membrane mechanisms (Hasenstaub, Shu, Ghandi, and McCormick, unpublished observations). Nevertheless, the possibility that intrinsic mechanisms, such as plateau potentials, determine the long time constants associated with the local UP states is being actively investigated (Cossart, Aronov, and Yuste, unpublished). Intracellular recordings *in vivo* from awake animals (Brecht et al. 2004), or the analysis of spike trains during delayed memory tasks, may help to resolve the issue of how this persistent activity is generated.

POSSIBLE CONTRIBUTION OF NEURONAL BARRAGES TO ATTENTION MECHANISMS

Attention to specific regions of sensory space, or specific features of an object, can be rapidly shifted at will or in response to a stimulus and results in a significant increase in signal detectability (e.g., salience) and decrease in reaction times. Extracellular recordings in the visual system often reveal attention to be associated with an increase in neuronal responsiveness, especially to less salient stimuli (such as a low-contrast grating) (Reynolds et al. 2000; McAdams and Maunsell 1999). Through what mechanisms could attention result in a rapid change in the excitability of individual as well as larger groups of cortical neurons? The release of neuromodulatory agents, such as acetylcholine or norepinephrine, is unlikely to be responsible since these have far too slow a time course to underlie the rapid changes in excitability associated with shifts in attention. The leading hypothesis is that attentional mechanisms involve rapid reconfiguration of neuronal networks through shifts in the synaptic bombardment of key elements of the network that corresponds to the stimulus region or feature that is being attended to. Our results suggest that increasing the synaptic bombardment of a cortical cell with a depolarizing barrage of EPSPs and IPSPs may result in enhancements of neuronal excitability that are similar to those observed in some attentional paradigms (Reynolds et al. 2000). These shifts in responsiveness are naturally stronger for weak, versus strong stimuli (see Figure 16.7).

Attention can also result in an increase in neuronal "gain" for all stimuli, meaning that the spike rate output for each stimulus is increased by the same percentage, regardless of the magnitude of the input (McAdams and Maunsell 1999). How might such an increase in neuronal gain be achieved? It has been proposed that the rapid removal of barrages of synaptic potentials may underlie attentional changes in gain: if the incoming PSPs are perfectly balanced between excitation and inhibition, then there will be no net change in membrane potentials, and the reduced neuronal conductance will make the cell more responsive to its other inputs (Chance et al. 2002). However, this proposal has two unusual features. First, it implies that the neurons representing all of the large parameter space that is unattended are constantly bombarded with synaptic activity so as to keep their responsiveness low. Second, this model of attention requires a precise and ongoing balance of incoming EPSPs and IPSPs so that the membrane potential of the cell is unaffected by changes in background PSP rate (even though the membrane potential itself is constantly changing). It is difficult to imagine exactly how this precise balance between EPSPs, IPSPs, and membrane potential, even as it fluctuates, could be obtained so precisely. Rather, we propose that the network dynamics of neurons representing the attended object or attended spatial location are altered to facilitate a "pop-out" effect (Figure 16.8). We envision a dynamic interaction of a facilitatory attentional template of the attended object with the neurons that represent that object leading to an

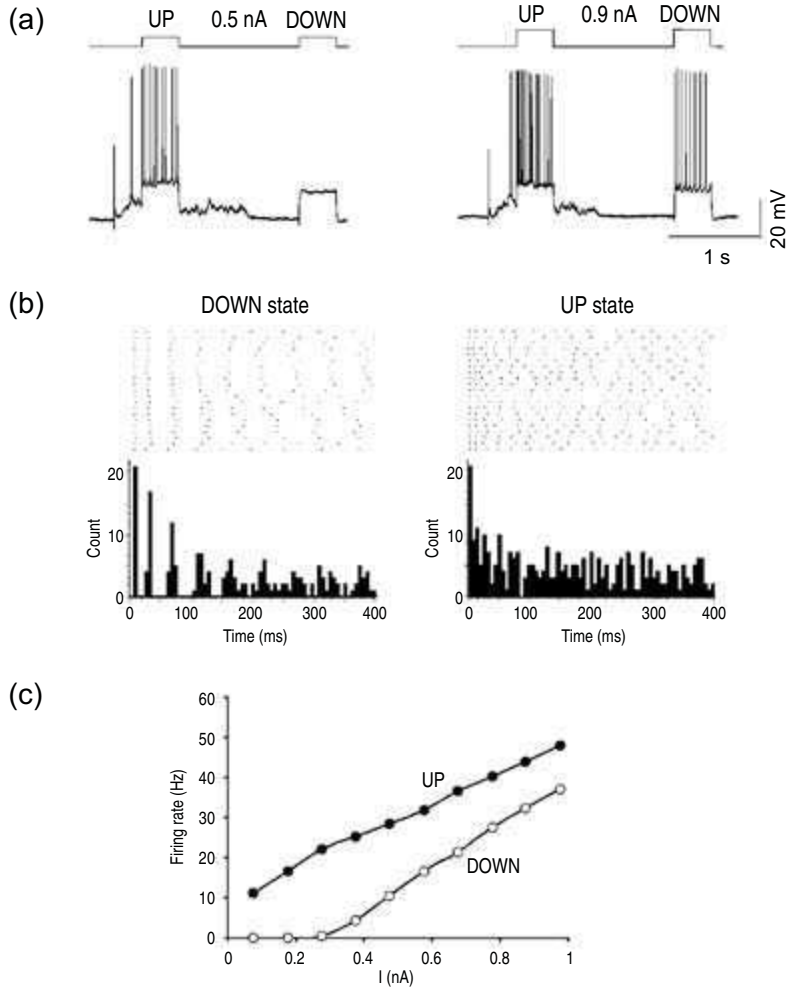

Figure 16.7 The UP state is associated with a marked increase in neuronal excitability. (a) Intracellular injection of depolarizing current pulses during the UP and DOWN states reveal a marked increase in neuronal responsiveness during the UP in comparison to the DOWN state. Two different amplitudes of current pulses are illustrated. (b) Stimulus histograms of the action potential (AP) response of the cell to repeated injections (I) of the same current pulse (0.7 nA) in the DOWN and UP states. The cell responds with more APs in the UP state, and the timing of these APs varies from pulse to pulse. (c) Response of the neuron to different amplitude current pulses in the UP and DOWN states. Note the marked increase in neuronal responses, especially to smaller amplitude inputs. From McCormick et al. (2003).

increase in response to the attended object. This results in an increase in its apparent salience in a manner similar to that recently proposed with computational models (Hahnloser et al. 2002). Finally, it is also possible that intrinsic cellular

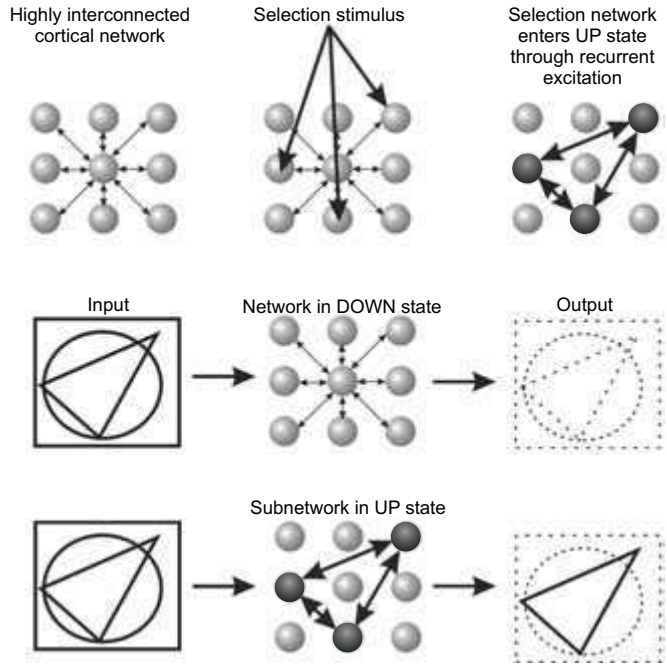

Figure 16.8 Hypothetical involvement of synaptic barrages and recurrent excitation in the facilitatory effects of selective attention. In a highly interconnected network of cells, many configurations are possible. A "top-down signal" may select a configuration of cells that roughly matches the form that is being attended. These cells may go into the UP state (or a similar depolarized state) through lateral interactions in response to the attentional signal. The arrival of a complex input to the network will then result in the selective transfer of information from neurons representing the attended signal, if the subnetwork is in the UP state. In the DOWN state, cells are equally unresponsive (dashed lines in middle, right).

mechanisms contribute to or interact with synaptic mechanisms of rapid changes in neuronal excitability. These intrinsic mechanisms may facilitate or control all aspects of neuronal control of neuronal excitability, including spike timing, spike initiation, and dendrosomatic coupling. The interactions of intrinsic and network functions remain to be explored.

SUMMARY

Our results demonstrate that both global or local cortical networks have the ability to generate recurrent periods of relatively high firing rate activity, which in some cases could be mediated by precisely balanced increases in both recurrent excitation and inhibition. UP states can be considered an emergent feature of circuit attractors resulting from recurrent excitatory connections. The spontaneous

recurrence of periods of locally generated UP states interspersed with periods of relative inactivity (DOWN) states resembles the transition between states observed *in vivo* during slow wave sleep and anesthesia. Although UP and DOWN states have not been clearly studied in the waking cortex, we hypothesize that similar mechanisms may underlie rapid changes in neuronal activity or excitability during behavior. The generation of local recurrent activity may be used to form temporary "memories" or to control neuronal responsiveness—computationally advantageous modifications that may serve multiple functions in the brain.

CONTROVERSIES, UNSOLVED PROBLEMS, OPEN QUESTIONS

- Do UP and DOWN states occur in waking animals? If not, is there activity that is similar to these (e.g., recurrent excitation that reverberates through a network to simultaneously affect them, such as with depolarization)?
- Is the ongoing background activity found in the waking brain similar to an UP state occurring during sleep? What is the role of neuromodulatory transmitters in maintaining the steady depolarization of the waking brain?
- How are locally sparse UP-like states generated and how do they maintain their sparse local neuronal participation in comparison to locally dense participating networks?
- Is attention associated with an increase in synaptic barrage of the affected neuronal networks, or a decrease?
- What is the interaction of top-down and bottom-up influences in determining neuronal responses?
- Does the background level of depolarization in the cortical network convey the psychological and experiential context and bias of the person?
- Is persistent activity *in vivo* generated through recurrent networks exclusively or are intrinsically generated mechanisms important?
- How does the cortical network generate maintained activity in the face of synaptic depression? Is synaptic depression *in vivo* really that prominent? Do *in vitro* techniques overemphasize its amplitude and properties?
- Can the cortex generate multiple levels of depolarization during UP-like states? Or is the activity more all-or-none?

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REFERENCES

Anderson, J., I. Lampl, I. Reichova, M. Carandini, and D. Ferster. 2000. Stimulus dependence of two-state fluctuations of membrane potential in cat visual cortex. *Nat. Neurosci*. **3**:617–21.

Brecht, M., M. Schneider, B. Sakmann, and T.W. Margrie. 2004. Whisker movements evoked by stimulation of single pyramidal cells in rat motor cortex. *Nature* **427**: 704–10.

Chance, F.S., L.F. Abbott, and A.D. Reyes. 2002. Gain modulation from background synaptic input. *Neuron* **35**:773–782.

Compte, A., M.V. Sanchez-Vives, D.A. McCormick, and X.-J. Wang. 2003. Cellular and network mechanisms of slow oscillatory activity (<1 Hz) and wave propagations in a cortical network model. *J. Neurophysiol.* **89**:2707–2725.

Constantinidis, C., M. Franowicz, and P. Goldman-Rakic. 2001. Coding specificity in cortical microcircuits: A multiple-electrode analysis of primate prefrontal cortex. *J. Neurosci.* **21**:3646–3655.

Cossart, R., D. Aronov, and R. Yuste. 2003. Attractor dynamics of network UP states in the neocortex. *Nature* **423**:283–288.

Cowan, R.L., and C.J. Wilson. 1994. Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. *J. Neurophysiol.* **71**:17–32.

Egorov, A.V., B.N. Hamam, E. Fransen, M.E. Hasselmo, and A.A. Alonso. 2002. Graded persistent activity in entorhinal cortex neurons. *Nature* **420**:173–178.

Fuster, J.M. 1995. Memory in the Cerebral Cortex. Cambridge, MA: MIT Press.

Goldman-Rakic, P.S. 1995. Cellular basis of working memory. *Neuron* **14**:477–485.

Hahnloser, R.H.R., R.J. Douglas, and K. Hepp. 2002. Attentional recruitment of inter-areal recurrent networks for selective gain control. *Neural Comp.* **14**:1669–1689.

Hô, N., and A. Destexhe. 2000. Synaptic background activity enhances the responsiveness of neocortical pyramidal neurons. *J. Neurophysiol.* **84**:1488–1496.

Hopfield, J.J. 1982. Neural networks and physical systems with emergent collective computational abilities. *PNAS* **79**:2554–2558.

Leopold, D.A., Y. Murayama, and N.K. Logothetis. 2003. Very slow activity fluctuations in monkey visual cortex: Implications for functional brain imaging. *Cereb. Cortex* **13**:422–433.

Mao, B.Q., F. Hamzei-Sichani, D. Aronov, R.C. Froemke, and R. Yuste. 2001. Dynamics of spontaneous activity in neocortical slices. *Neuron* **32**:883–898.

McAdams, C.J., and J.H.R. Maunsell. 1999. Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. *J. Neurosci.* **19**:431–441.

McCormick, D.A., Y. Shu, A. Hasenstaub et al. 2003. Persistent cortical activity: Mechanisms of generation and effect on neuronal excitability. *Cereb. Cortex* **13**:1219–1231.

Reynolds, J.H., T. Pasternak, and R. Desimone. 2000. Attention increases sensitivity of V4 neurons. *Neuron* **26**:703–714.

Salinas, E., and T.J. Sejnowski. 2001. Correlated neuronal activity and the flow of neural information. *Nat. Neurosci. Rev.* **2**:539–550.

Sanchez-Vives, M.V., and D.A. McCormick. 2000. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat. Neurosci.* **3**:1027–1034.

Seamans, J.K., L. Nogueira, and A. Lavin. 2003. Synaptic basis of persistent activity in prefrontal cortex *in vivo* and in organotypic cultures. *Cereb. Cortex* **13**:1242–1250.

Shadlen, M.N., and W.T. Newsome. 1998. The variable discharge of cortical neurons: Implications for connectivity, computation, and information coding. *J. Neurosci.* **18**:3870–3896.

Shu, Y., A. Hasenstaub, M. Badoual, T. Bal, and D.A. McCormick. 2003b. Barrages of synaptic activity control the gain and sensitivity of cortical neurons. *J. Neurosci.* **23**:10,388–10,401.

Shu, Y., A. Hasenstaub, and D.A. McCormick. 2003a. Turning on and off recurrent balanced cortical activity. *Nature* **423**:288–292.

Silberberg, G., A. Gupta, and H. Markram. 2002. Stereotypy in neocortical microcircuits. *TINS* **25**:227–230.

Stériade, M., A. Nunez, and F. Amzica. 1993. A novel slow (<1 Hz) oscillation of neocortical neurons *in vivo*: Depolarizing and hyperpolarizing components. *J. Neurosci.* **13**:3252–3265.

Stériade, M., I. Timofeev, and F. Grenier. 2001. Natural waking and sleep states, a view from inside neocortical neurons. *J. Neurophysiol.* **85**:1969–1985.

Super, H., C. van der Togt, H. Spekreijse, and V.A. Lamme. 2003. Internal state of monkey primary visual cortex (V1) predicts figure-ground perception. *J. Neurosci.* **23**:3407–3414.

Timofeev, I., D. Contreras, and M. Stériade. 1996. Synaptic responsiveness of cortical and thalamic neurons during various phases of slow sleep oscillation in cat. *J. Physiol.* **494**:265–278.

Wang, X.J. 2001. Synaptic reverberation underlying mnemonic persistent activity. *TINS* **24**:455–463.

Wilson, C.J., and P.M. Groves. 1981. Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. *Brain Res.* **220**:67–80.

Wilson, H.R., R. Blake, and S.H. Lee. 2001. Dynamics of traveling waves in visual perception. *Nature* **412**:907–910.

17

Mechanisms of Neural Integration at the Brain-scale Level

The Neuronal Workspace and Microstate Models

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ABSTRACT

Plausible, but still largely hypothetical, neural architectures and physiological model processes are reviewed that may contribute to the integration of microcircuits (or ensemble of microcircuits) at the brain-scale level. First, a distinction is proposed between a set of parallel, distributed, and functionally encapsulated processors and a global workspace assumed to consist of a distributed set of cortical neurons which physically integrate the multiple processors from distinct areas and/or hemispheres by their long-range excitatory axons. In parallel, careful analysis of "global" EEG recordings reveal a striking segmentation of the EEG activity into discrete "functional microstates." It is proposed that the temporal organization of these microstates reflects the dynamics of the conscious content of the neuronal workspace. Both approaches underline the top-down contribution of ongoing spontaneous activity to the all-or-none access of sensory stimuli to "consciousness." Last, a model for reward-dependent selection of representations within the neuronal workspace is presented which allows a rapid and closer fit of the brain with its environment.

INTRODUCTION

With the elucidation of the complete sequence of the human genome, all molecular species composing the human body and, in particular the brain, are known, or should soon be known. Ultimately, from the DNA sequences stored *in silico*,

one should be able to compute the main features of the species-specific functional organization of our brain. Yet, if we are still far from it, the underlying research program is clear and intensive research of developmental molecular biology is underway (see Changeux 2005a). At the organism level, the rapid progress of cognitive sciences, integrative physiology, and brain imaging offers a rich repertoire of behaviors and/or internal computations that determine the plans of action of the organism on the physical and social world together with their execution as defined motor actions, including the use of language.

It thus appears urgent to definitively abandon the "dualist" position (see Eccles 1989), which maintains a cleavage between these extreme levels and bypasses the multiple and intricate levels of organization of the underlying molecular and cellular networks. The challenge of the twenty-first century in neuroscience is to establish the causal relationships existing, through the hierarchically nested levels of organization, between the molecular–cellular–microcircuit level (the focus of this Dahlem Workshop) and the actual mobilization of distributed ensembles (or "coalitions") of neurons that determine the behavior of the organism. The issue is both theoretical and experimental. It is to look for neural architectures and processes that unite or integrate parts or elements at a given level of organization into larger units at an immediately higher level. Selected examples in this increasing hierarchy are: the genesis of single-cell firing from intrinsic pacemakers and synaptic responses, the building up of coherent distributions of activity commanding fixed action patterns up to the "conscious mental syntheses" for problem solving, planning, and decision making.

The aim of this brief review is to present and discuss plausible, but still largely hypothetical, neural mechanisms that integrate these microcircuits (or ensemble of microcircuits) into higher functional entities or "global" brain-scale mental objects. Plausible neuronal architectures (Dehaene, Kerszberg, and Changeux 1998; Dehaene et al. 2003; Dehaene and Changeux 2005) are proposed which, at the highest level of integration, create a "conscious workspace" (Baars 1988) involved in mental syntheses and decision making. Independently, electrophysiological recordings at the brain-scale level (Lehmann et al. 1995, 1998; Michel et al. 1999) have revealed an unexpected phenomenon: the segmentation of the global brain activity into discrete temporal units, referred to as "functional microstates." These microstates are interpreted as reflecting the dynamics of the flow of the conscious content within the neuronal workspace. It will also be shown that access of sensory stimuli to consciousness relies on the brain's, up to now rather neglected, ongoing spontaneous activity, which reflects the momentary global brain state. Finally, we will mention that reward-dependent neuronal learning processes may give rise to selection of representations (Dehaene and Changeux 2000) within the neuronal workspace, thus establishing what may be referred to as an adequacy between the internal neural organization and the outside world; in other words, the integration of the organism into its physical, social, and cultural environment.

Several of these issues have been recently reviewed (Changeux 2005a, b; Dehaene and Changeux 2000, 2004, 2005; Dehaene, Kerszberg, and Changeux 1998; Dehaene et al. 2003; Lehmann et al. 1995, 1998; Michel et al. 1999; Changeux and Edelstein 1998).

THE NEURONAL WORKSPACE HYPOTHESIS AND THE MECHANISMS FOR ACCESS TO CONSCIOUSNESS

Definitions

In higher vertebrates and humans, consciousness may be viewed as the ultimate level of brain integration and *bona fide* assumed to be caused by neurobiological processes and realized in brain structures (see Changeux 1983, 2005a, b; Edelman 1987; Crick 1994; Koch 2004). Being awake, we "live" the subjective experience of a unified or global field or scene, where some kind of synthesis between past, present, and future takes place. Multimodal exchanges of perceptions (present), emotions and feelings, evoked memories of prior experiences (past), together with anticipations of actions (future) become subjectively integrated resulting in a continuously changing and dynamic flow of consciousness, "altogether one and multiple at any of its moments" (James 1890; Fessard 1954; Tononi and Edelman 2000; Crick and Koch 2003; Koch 2004). Yet, the context of consciousness is so broad and the issues often so muddled that scientific investigations on this subject have to be restricted to empirically tractable issues that are accessible to both experimental measurements and formal modeling in terms of neuronal networks.

Before entering the debate, one should distinguish what may be referred to as a state of consciousness in contrast to a content of consciousness. The states of consciousness include awake state, sleep, dream, and coma, but also passive (or perceptual) consciousness as opposed to an attentive and reflexive consciousness including planning, reasoning, and efforts to solve problems with intense introspection (Bergson 1959; Taylor 1999). Among the neurophysiological correlates of the diverse states of consciousness, the low-frequency rhythmic activities (<15 Hz) that characterize slow-wave sleep and the occurrence of fast rhythms (20–50 Hz) that define waking and REM sleep are viewed as being generated in interconnected loops established between neurons from thalamus and cerebral cortex. The transitions between these states are under the control of generalized modulatory systems, among them cholinergic and noradrenergic (Stériade 2003; Jones 1998; Hobson 1999; Llinas and Ribary 1998; Pau 2000; Dehaene and Changeux 2005). For instance, stimulation of nicotinic receptors (Changeux and Edelstein 2005), which are abundant in thalamic and cortical neurons (Léna and Changeux 1997), increases wakefulness and attention (Levin and Rezvani 2002), and the deletion of the $\alpha 2$ subunit from high-affinity

($\alpha4\beta2$) neuronal nicotinic receptor shows a deficit in awakening condition (Cohen et al. 2002; Léna et al. 2004) as manifested by a decrease of "microarousals" occurring during slow-wave sleep (Léna et al. 2004). Furthermore, constitutive "gain of function" mutations known to alter the allosteric properties of neuronal nAChR (in the $\alpha4$ and/or $\alpha2$ subunit) (Changeux and Edelstein 1998, 2005) cause nocturnal frontal lobe epilepsies (Steinlein et al. 1995; Steinlein 2004); these crises occur during light slow-wave sleep precisely at the time when microarousals occur (Sutor and Zolles 2001).

The content of consciousness, by contrast, refers to the temporary selection of a well-delimited category of global representation, which is made potentially available to a broad variety of neural processes, thus giving rise to a subjective feeling but also to a "report" of conscious access by the subject. The neuronal workspace hypothesis (Dehaene, Kerszberg, and Changeux 1998) that will be briefly summarized below only deals with the "content" of consciousness.

Distinction between Conscious and Nonconscious Processings

During sleep and deep general anesthesia the subject is nonconscious with notable, though limited, recall of the dreaming episodes. Even when awake, alert subjects may not be aware that they are carrying out intense nonconscious processing. In so-called "blind sight," a lesion of the primary visual area 17 causes a loss of elementary visual capacity in defined regions of the visual field. Nevertheless, if a light flash is presented in the blind field of the patient and one asks him to move the eyes (Poppel et al. 1973) or to point with a finger in the direction of the flash (Weiskrantz 1997), the patient does it correctly, denying that he ever saw any light flash. This blind sight reveals the contribution of cerebral cortex processing to conscious vision and an efficient transfer of information through noncortical pathways without the subject being aware of it.

Another line of experimental evidence in favor of nonconscious processing (among many others) is the phenomenon of semantic priming (Dehaene, Naccache et al. 1998; reviewed in Dehaene and Naccache 2002). Visual words that are masked are presented to subjects so briefly that according to the report of the subject they cannot be perceived consciously. Nevertheless, they facilitate the subsequent processing of related words by the same subject. Brain imaging techniques reveal that briefly unmasked stimuli have a measurable influence on brain activation patterns, in particular in the areas involved in motor programming, which are covertly activated. Moreover, these patterns differ dramatically when the subject, according to his reports, sees the stimulus in a fully conscious compared to a nonconscious manner (Dehaene, Naccache et al. 1998).

The Neuronal Workspace Hypothesis

The neuronal workspace model (Figure 17.1) first formulated by Dehaene, Kerszberg, and Changeux (1998) (see also Dehaene et al. 2003; Dehaene and

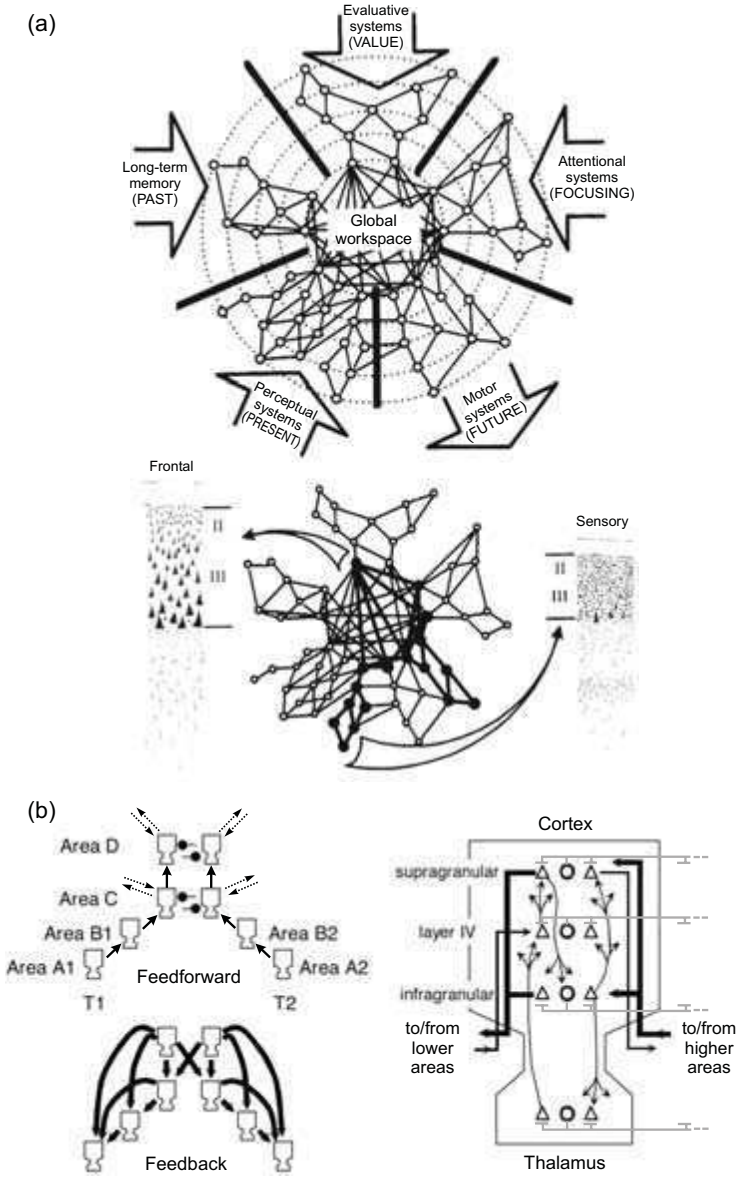

Figure 17.1 Schematic representation of the neuronal workspace hypothesis. (a) The principal automatic processors are represented together with the global—conscious—workspace composed of layers II–III pyramidal neurons with long-range axons, links between processor neurons become established through the activation of distributed workspace neurons. (b) Application of the workspace model to the all-or-none access to consciousness: feedforward and feedback connectivity give rise to threshold in the processing of sensory entries. Source: Dehaene, Kerszberg, and Changeux (1998) and Dehaene et al. 2003.

Naccache 2002; Dehaene and Changeux 2005) aims to account for the conscious versus nonconscious processing in simple neurological terms. It is an attempt to implement altogether the independent nonconscious processing of a large diversity of signals in distinct parallel pathways and their global integration within a "unitary field" (Searle 2000) or psychological conscious "workspace" (Baars 1988).

The central proposition of the hypothesis is the neural distinction in the brain of two main computational spaces. The first is a processing network composed of parallel, distributed, and functionally encapsulated processors organized from cortical microcircuits. These processors range from primary (or even heteromodal) sensory processors, motor processors, long-term memory stores including semantic database, the self, autobiographical and personal data, attentional and evaluative systems including motivation, reward, and, in general terms, the emotions. The second computational space is referred to as a global workspace and it is assumed to consist of a distributed set of cortical neurons, which physically integrate the multiple processors by their ability to receive from and send back to homologous neurons in other cortical areas through long-range excitatory axons. These horizontal projections interconnect at the brain-scale distant areas in the same hemisphere and between hemispheres through the corpus callosum. The early observations of Cajal (1909) and Von Economo (1929), which are supported by a large body of recent observations (see Mountcastle 1998), indicate that pyramidal cells from layers II and III of the cerebral cortex (among others) possess long axonal processes that they send within and between hemispheres. They are postulated to contribute to the neural workspace in a privileged manner (a similar hypothesis has been proposed, yet in a different context [cortical associative memory] by Fransén and Lansner 1998 [see Frégnac et al., this volume]). Yet horizontal connections linking thalamic nuclei may also be part of the neuronal workspace. An important consequence of these postulated neural architectures, already noticed by Von Economo (1929), is that pyramidal neurons of cortical layers II and III are especially abundant in dorsal lateral prefrontal and inferoparietal cortical areas thus offering a brain-scale regional correlate of this cellular hypothesis in terms of a topology of activated cortical areas. As a consequence these selective contributions might be directly evaluated by brain imaging techniques.

The model posits that, in a conscious effortful and attentive task, workspace neurons become spontaneously coactivated forming discrete though variable spatiotemporal patterns of activity, some kind of global prerepresentation. Such "brain-scale" representations would mobilize neurons from multiple brain processors in a reciprocal manner and be subject to regulation by vigilance and attention neuromodulators and to selection by reward signals (see section on REWARD-DEPENDENT LEARNING). Their eventual recording as "functional microstates" in the EEG is discussed in the section on FUNCTIONAL "MICROSTATES" OF THE BRAIN.

The relationships postulated between the workspace and processor neurons are reciprocal and both top-down and bottom-up, albeit not symmetrical. Processor neurons—like those which mediate sensory inputs—project, in a bottom-up manner, to the interconnected set of neurons composing the global workspace. Conversely, at any given time, the global representations of the workspace selectively gate in a top-down manner only a subset of processor neurons. The hypothesis posits that this top-down control is mediated by descending modulatory projections from workspace neurons to more peripheral processor neurons (which could either be cortical or thalamic or both) (see Lumer et al. 1997). These projections may selectively amplify, or extinguish, the ascending inputs from processing neurons, thus mobilizing, at a given time, a specific set of processors in the workspace, while suppressing the contribution of others (for neural implementation, see Dehaene, Naccache et al. 1998; Dehaene et al. 2003).

A prerepresentation that has been selected as "global representation" within the workspace may remain active, in an autonomous manner, and resists changes in peripheral stimulation as long as it receives positive reward signals. If negatively evaluated or if the self-sustained processes of attention fail, it may be updated or replaced through trial-and-error processing by another discrete combination of workspace neurons through reward-dependent selection mechanisms (see section on REWARD-DEPENDENT LEARNING).

These principles have been applied to a well-known test of frontal function, the Stroop task (Dehaene, Naccache et al. 1998). This test comprises both an easy task (naming a color word) and a difficult task (naming the color of the ink in which a word is printed, when the word itself is the name of an incompatible color; for example, saying "blue" when seeing the word "green" printed in blue ink). The computer simulations show that the model network passes the easy word naming task without needing to activate any workspace units. When the model is switched to the more difficult Stroop task, workspace activation initially increases during a search phase in which acquisition of the task is accompanied by an intense and highly variable activation of workspace units. This search phase ends when a workspace activation pattern is found, which leads to successful performance of the task.

The search phase is followed by an effortful execution phase, during which the workspace neurons remain in a stable state of high activity; progressively, vigilance decreases as the task becomes routinized and is transferred, through synaptic modifications, to the processor units and their interconnections. Following routinization, workspace neurons' activation is no longer needed.

Brain-imaging experiments indicate that, in agreement with the neuronal workspace hypothesis, dorsolateral prefrontal cortex (dlPFC) and anterior cingulate (AC) (which are particularly rich in layer II/III long axon neurons) are active in effortful cognitive tasks, including the Stroop test, with a graded level of activation as a function of task difficulty (Pardo et al. 1990; Cohen et al. 1997;

Paus et al. 1998). Electrophysiological recordings further suggest that this increased frontal blood flow actually reflects a prolonged activity of a functional microstate (see section on FUNCTIONAL "MICROSTATES" OF THE BRAIN) at around 300 ms (Khateb et al. 2000). This is directly consistent with the idea of a prolonged stable state of high activity during the effortful execution phase. With automatization, activation decreases in dlPFC and AC, but it immediately recovers if a novel, nonroutine situation occurs (Raichle et al. 1994).

The Neuronal Mechanisms for Access to Consciousness

An important consequence of the neuronal workspace hypothesis is that the step of conscious perception, referred to as access awareness, would be related to the entry of processed visual stimuli into the global brain state that links distant areas, including the prefrontal cortex, through reciprocal connections and thus makes perceptual information reportable by multiple means. An extension of the neuronal workspace model has been recently proposed to simulate a classical perceptual phenomenon: the attentional blink (Dehaene et al. 2003). In a typical experiment, subjects are asked to process two successive visual targets, 1 (T1) and 2 (T2). When T2 is presented between 100 and 500 ms after T1, the ability to report it drops, as if the participants' attention has "blinked." The processed signal is no longer "conscious." Objective physiological data indicate that during this blink, T2 fails to evoke a P300 electrical potential but still elicits event-related potentials associated with visual and semantic processing (P1, N1, and N400). The simulations illustrate how some patterns of brain activity become selectively associated with subjective experience while others do not. In short, during the blink, bottom-up activity, presumably generating the P1, N1, and N400 waveforms, would propagate without necessarily creating a global reverberant state. However, a characteristic neural signature of long-lasting distributed activity and gamma-band emission, presumably generating the P300 waveform, would be associated with global access.

The elementary components of the simulation are single-compartment spiking neurons organized in schematic cortical columns reciprocally connected to a thalamic network that receives external inputs. When depolarized beyond a certain threshold by neuromodulatory inputs, these neurons exhibit a spontaneous activity through intrinsic membrane oscillations in the γ-range distinct from the activity evoked by sensory stimulation (Dehaene and Changeux 2005).

In agreement with the neural workspace model and with anatomical and physiological models of cortical organization, a nearest-neighbor, bottom-up network that propagates sensory stimulation across the hierarchy of areas is distinguished from a long-distance, top-down network that sends amplification signals back to all levels below it.

A possible neurochemical implementation of the model network, among others, is that bottom-up propagation mobilizes fast glutamatergic α-amino-3-

hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, whereas top-down amplification is mediated by slower glutamatergic N-methyl-D-aspartate (NMDA) receptors. These top-down projections are crucial in the model: they exert a modulatory role on the efficacy of voltage-gated NMDA receptors, which is enhanced (possibly through Mg^{2+} unblocking of the ionic channel) upon concomitant depolarization by sensory inputs. They are necessary for the regenerative nonlinear processing of the sensory inputs entering the workspace.

Thus, access to the workspace, in other words global activation, is achieved when there is "resonance" between bottom-up sensory information and top-down spontaneous signals (an issue further discussed in the next section as "state-dependent information processing").

The model reproduces the main objective and subjective features of the attentional blink paradigm. In addition, it predicts a unique aspect of information processing in the brain: the nonlinear transition from nonconscious processing to conscious subjective perception. This all-or-none dynamics of conscious perception has been verified with human subjects performing blink tasks (Sergent and Dehaene 2004). It is a characteristic feature of a typical integrative mechanism at the highest cognitive level.

FUNCTIONAL "MICROSTATES" OF THE BRAIN

The neuronal workspace hypothesis posits that in the course of the performance of an effortful cognitive task (such as the Stroop task or the blink test) spontaneous coactivations of workspace neurons take place at the brain-scale level. These discrete episodes of coherent activity last a certain amount of time and are separated by sharp "nonlinear" transitions. Only one such workspace representation is active at any given time (Dehaene, Kerszberg, and Changeux 1998; Dehaene et al. 2003).

The hypothesis is now suggested that this consequence of the workspace model adequately fits the experimental "functional microstates" notion initially proposed on the basis of EEG recordings (e.g., Lehmann 1980, 1990, 1992; Michel et al. 1999, 2001). Indeed, the analysis, in conscious and attentive subjects, of a large number of recordings of multichannel EEG and event-related potentials revealed that, as time elapses, the topography of the scalp electric fields does not change randomly and continuously. On the contrary, the configuration of the global electric field (the landscape) remains stable for a certain length of time (~80–150 ms), then very quickly changes into a new configuration in which it remains stable again. There is no continuous smooth transition from one landscape to the other but discrete segments of electrical stability separated by sharp transitions. Within a given time episode, the strength of the field varies but the topography remains stable (Figure 17.2). This observation led to the hypothesis that these episodes of stable electric field configurations reflect

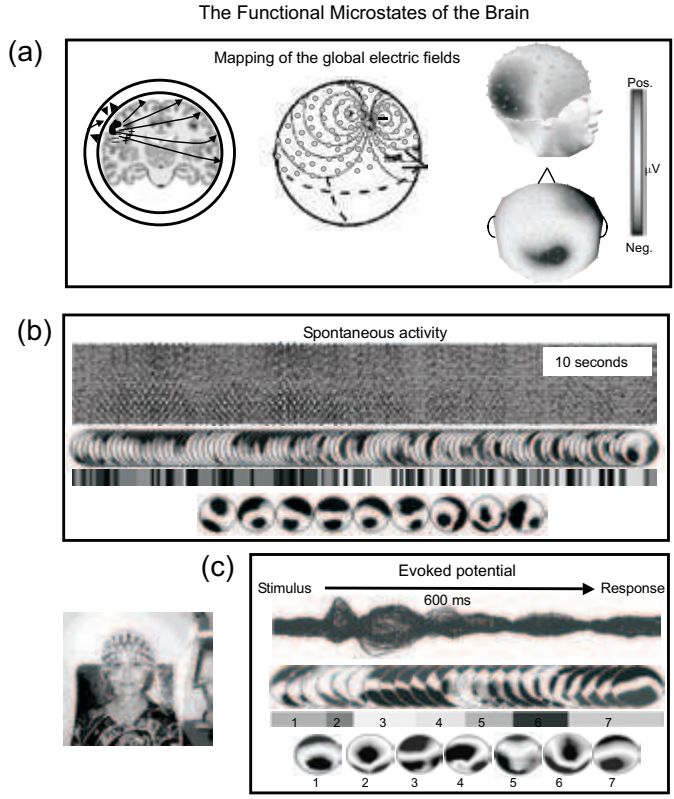

Figure 17.2 Assessment of the functional microstates of the brain. (a) Synchronous activity of a large number of neurons in a given cortical region leads to a sum electric field that spreads throughout the brain and reaches the cortical surface. By placing a large number of electrodes on the scalp surface (here: 128), the instantaneous electric field can be recorded directly and displayed as a scalp potential map in 3D or as a planar projection. (b) 10 seconds of spontaneous EEG recorded from 128 electrodes. Below some selected traces, a series of scalp potential maps is shown (note than only a subsampling of the total 5000 maps is shown). The 9 maps at the bottom are the result of a k-means spatial cluster analysis that determined the most dominant map configurations present in the data. The bar below the map series indicates the periods during which each of these maps was present. The striking finding is that the electric activity can be divided into time segments of around 80–150 ms duration that are each characterized by a stable map configuration. It is believed that each of these periods represents a functional microstate of the brain, that is, an "atom of thought." (c) Brain electric responses evoked by a stimulus (a word in this example) simultaneously recorded from 128 scalp electrodes. The evoked potentials for the 600 ms post-stimulus are shown as overlaid traces. Below them, the map series during this period is shown (again only a subsample). The spatial cluster analysis revealed that 7 maps best explain the whole data set (shown on the bottom). The bar illustrates the period during which each of these 7 maps was present. Again, segments of stable map configuration, lasting between 80–150 ms are found. It is assumed that each of these evoked functional microstates represents a given information-processing step.

particular "steps" or "contents" of information processing; that is, they are the basic building blocks of the content of consciousness: "atoms of thoughts" (Koukkou and Lehmann 1987; Lehmann 1992) or "mental objects" (Changeux 1983), therefore the term "functional microstate" (note that "micro" here refers to the temporal not the spatial scale). According to this interpretation, what William James (1890) referred to as the "stream of consciousness" might not be continuous, but a sequence of separable, distinct, microstates that implement different mental contents. In keeping with the neuronal workspace model, we wish to suggest the hypothesis that the experimental functional microstates of the brain implement the postulated spontaneous or evoked coherent activation of workspace neurons. In other words, they would be electrophysiological correlates of a process of global "conscious" integration at the brain-scale level. They might also be viewed as implementing the proposal of Crick and Koch (2003) that, in the case of motion, perception consists of discrete processing epochs or "snapshots" as well as Oliver Sacks's clinical observations (2004) of patients who see movement as a succession of "stills" during migraine episodes.

These functional microstates have been systematically recorded in human subjects and analyzed in different "mental" conditions offering clues about the duration of what might be viewed as basic building blocks of the content of consciousness. A recent comprehensive analysis of 496 subjects between the age of 6 and 80 years revealed mean microstate durations of 80–150 ms (Koenig et al. 2002), confirming earlier studies with a smaller number of subjects (Wackermann et al. 1993; Lehmann et al. 1998). The duration decreases slowly during childhood and then stabilizes in adulthood (Koenig et al. 2002). According to Lehmann et al. (1998) these microstates would represent the elementary psychophysiological units of cognition and emotion. For instance, in an experiment where subjects were asked to recall spontaneous, conscious experiences after the presentation of a prompt signal, the reports could successfully be classified into imagery and abstract thoughts on the basis of the topography of the microstate just preceding the prompt. The duration of these microstates was on average 121 ms, indicating that approximately this duration of near-stable brain activity suffices for a conscious experience. Interestingly, cognitive event-related potential components, such as the CNV, the P300, or the N400, are characterized by periods of stable map topographies of approximately this duration (e.g., Michel et al. 1992; Pegna et al. 1997; Khateb et al. 2000, 2003; Schnider et al. 2002; Murray et al. 2004). Moreover, the more effortful the task process, the longer was the duration of the corresponding cognitive microstates (Pegna et al. 1997; Khateb et al. 2000). Other evidence has led to similar recordings of activity parcellation into sequential episodes of around 100 ms, making the functional microstates plausible candidates for the electrophysiological manifestation of these global episodes of conscious experience. For example, sequentially presented stimuli are not perceived as separate when they follow each other within less than 80 ms (Efron 1970). Masking a stimulus is efficient when

presented with a latency of less than 100 ms (Libet 1981; Dehaene et al. 2003; Sergent and Dehaene 2004). Other studies have reported similar durations for episodes of synchronous thalamo-cortical activity (Llinas and Ribary 1998), sequences of alpha bursts (Williamson et al. 1996), or of EPSP-IPSP in mammalian forebrain neurons (Purpura 1972; review in John 2002). Thus, the functional microstates, expressed as periods of stable scalp electrical potential topographies, may be viewed as neural implementations of the elementary building blocks of consciousness content. In line with this view is the recent demonstration of shortening of certain microstates in schizophrenics, which could functionally be interpreted as a precocious termination of information processing in certain classes of mental operations due to degraded cooperativity of neural assemblies in these patients (Lehmann et al. 2005).

STATE-DEPENDENT INFORMATION PROCESSING

The notion that the brain operates in a strictly bottom-up, input–output processing system is rather widespread. Still, strong evidence supports the opposite view that the fate of sensory inputs entering the brain depends on the actual functional state of the brain at or just before stimulus entry. This idea of a top-down control of brain functions underlies the functional state-shift hypothesis formulated by Koukkou and Lehmann (1983, 1987) and is consistent with the concept of selection of neural prerepresentations by resonance or reward (Changeux 1983, 2005b; Dehaene et al. 1987; Dehaene and Changeux 1989, 1991). Indeed, electrophysiological recordings revealed that spontaneous fluctuations of electric activity at the time of stimulus arrival control the way a stimulus is going to be perceived and processed.

EP Differences Based on Prestimulus EEG

The hypothesis that momentary functional microstate at stimulus arrival influences the way the stimulus is perceived and processed was tested directly by determining the most dominant microstate just before stimulus presentation and then calculating the stimulus-evoked potentials (EP) separately for these prestimulus classes. As anticipated, experiments with different types of visual or auditory stimuli revealed drastic EP differences at different latencies (Lehmann et al. 1994; Kondakor et al. 1995, 1997). They were not dependent on a particular cognitive task or a particular cognitive load, since state-dependent EP variations were recorded in a simple auditory odd-ball paradigm as well as in complex visual attention tasks. Recently, Braeutigam and Swithenby (2003) have described state-dependent evoked magnetic fields that were specific for the perception of faces. In a recent experiment, Mohr et al. (2005) found that the

functional state at the moment of stimulus entry influences functional hemispheric specialization for emotional word processing that differs between men and women. In a general manner, these findings suggest that subtle variations of ongoing functional state of the brain prior to input access provide an endogenous context that influences subsequent event-related information processing by the brain, which follows common rules over subjects (Lehmann et al. 1994).

Prestimulus Activation States Predict Performance

Electrophysiological recordings in monkeys show that the temporal structure of neuronal activity patterns which precede the rise of an evoked stimulus can be correlated with subsequent perceptual decisions or behavioral events (see Engel et al. 2001 for review). These patterns are often expressed as a synchrony in multiple site recordings of local field potentials: a synchrony that is dominant in the gamma frequency range. Using high-resolution EEG and direct estimation of local field potentials in humans, a positive correlation between gamma activity in a frontoparietal network before stimulus arrival and the reaction time to the stimulus was recently shown (Gonzalez Andino et al. 2004). It is assumed that these prestimulus synchronies reflect alerting attention mechanisms and the genesis of prerepresentations that influence stimulus processing through top-down control. These prestimulus differences in alerting attention might also explain the finding of Super et al. (2003) that the firing rate of V1 neurons 100 ms before the stimulus predicted whether or not the monkey consciously perceived a visual stimulus.

In summary, there exists consistent experimental evidence that subtle variations of the functional state of the brain which precede the entry of sensory stimuli influence subsequent event-related information processing and that some of them (like synchronies) may reflect alerting attention mechanisms. These data are in agreement with the critical proposal of the neuronal workspace model that access to consciousness relies upon the ongoing spontaneous activity of the workspace neurons. Still, further studies are needed to test the specific predictions of the formal model at the neuronal level (see Dehaene et al. 2003; Dehaene and Changeux 2005).

REWARD-DEPENDENT LEARNING

As outlined above, a given combination of workspace neurons will remain active autonomously as long as it is positively rewarded. If reward is missing, another discrete combination of workspace neurons may be activated. Possible mechanisms that underlie this reward-dependent processing will be discussed in this section. They may equally apply to lower-level patterns of neurons, for instance, in the striatum.

Definitions

The environment does not directly "instruct" the brain through evoked activity, as assumed by the standard empiricist and associationist schemes (Pavlov 1897; Kandel et al. 1995; Churchland and Sejnowski 1992). Adequation between the brain and its environment, in other words "knowledge acquisition," is viewed since Thorndike (1898), on the contrary, as indirect and resulting from the selection by trial and error (Holt 1931) of spontaneous variations, first at the gene level (biological evolution) but also in the brain at its multiple nested epigenetic levels (Changeux 1983; Edelman 1987). At the global brain-scale level, these neural variations of activity have been referred to as anticipations (Tolman 1951), prerepresentations (Changeux 1983), or preliminary neural schemes (Arbib et al. 1998) that take place spontaneously before or together with the interaction of the organism with the outside world. According to these views, the spontaneous activity of the brain would play a central role within some kind of Darwinian "generator of diversity" (Changeux 1983; Edelman 1987) in the production of these prerepresentations. The hypothesis has been suggested that these variable coherent patterns of neuronal activity (Faure and Korn 1997; Wackermann 1999; John 2002), exploit the reciprocal (Dehaene and Changeux 1989) or reentrant (Sporns et al. 1989) connections existing between excitatory cortical neurons (Hebb 1949), which together with networks of inhibitory neurons, mobilize, spontaneously and in a combinational manner (see Tsodyks et al. 1999), innate structures (such as diverse sensory modalities, and/or motor territories) as well as epigenetically stored forms (Changeux 1983).

The signal received from the external world will then determine if the initial prerepresentation is stabilized or not. It is a test for what may be called the saliency (McCarthy and Warrington 1990) or adequacy (Dehaene and Changeux 1989; Sporns et al. 1991) of the prerepresentation to the actual environment. Several classes of mechanisms have been suggested for such test of reality, among which the selection by reward for the evaluation of actions (Hull 1943). The signals received from the environment would, for instance, mobilize specialized neuronal pathways engaged in motivation and/or reward (Everitt et al. 2001), which may themselves be prone to learning (Sutton and Barto 1998; Schultz et al. 1997). Positive reward elicited from the outside world would result in the release of neuromodulatory substances (such as dopamine or acetylcholine or both) which would/or not stabilize the tested prerepresentation by changing the efficacies of the synapses linking the concerned neurons thus resulting in the storage of an adequate representation (review in Dehaene and Changeux 2000). Negative reward (or "punishment") would, on the opposite, destabilize the tested prerepresentation (Dehaene and Changeux 1989, 1991; Sporns et al. 1991; Tononi et al. 1992; Miyashita and Hayashi 2000).

In all instances, the information about rewards must be available to all synaptic sites at which reward-dependent plasticity is needed. This implies that the reward signal must be communicated by widely distributed neuromodulatory

projections possibly in a "volume" or paracrine fashion. This is consistent with the well-known widespread distribution of neuromodulatory dopaminergic, noradrenergic, serotoninergic, and cholinergic projections, which thus offer global mechanisms of integration at the brain scale.

Implementation, Test, and Limitation of the Hypothesis

Minimal architectures of formal neuronal networks capable of performing simple cognitive tasks (Dehaene and Changeux 1989, 1991, 1997; Dehaene, Naccache et al. 1998) have been built for delayed-response tasks (Wisconsin card sorting task, Tower of London), which were developed initially to test the ability of monkeys (or other species) and human patients to solve problems (Jacobsen 1935; review in Dehaene and Changeux 1989; Figure 17.3). For all of them, performance relied on prefrontal cortex integrity.

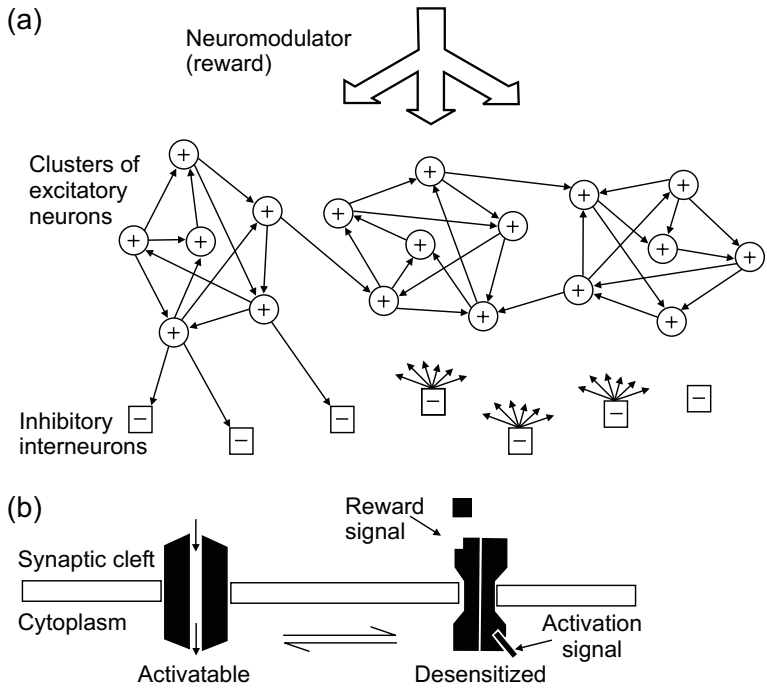

Figure 17.3 A neuronal model of a formal organism able to pass delayed-response tasks (a) and of learning by reward at the synaptic level (b). Schematic neuronal architecture with several nested levels of organization, each circle represents specialized groups of neurons. Synaptic mechanism for selection of rule neurons by reward: the hypothesized plasticity mechanism makes use of the integrative allosteric properties of a neurotransmitter receptor (from Dehaene and Changeux 1989, 1991).

Control of Synaptic Efficacy by Reward Signals

In standard neural networks, Hebbian learning, or backpropagation, synaptic efficacy is regulated strictly at the local level, such as recent pre- and postsynaptic activity. In reinforcement learning, an additional "global" signal coding for recent rewards is postulated to control the synaptic change. For instance (Dehaene and Changeux 1989, 2000), the simulations have utilized a simple rule:

$$\Delta w = \varepsilon S_{pre}\left(2S_{post} - 1\right)R,$$

where w is the synaptic weight, S_{pre} and S_{post} are the recent presynaptic and postsynaptic activities (between 0 and +1), and R is the reward (between –1 and +1). When the reward is positive, this equation gives the classical Hebb's rule and recent activations are stabilized. When the reward is negative, the rule becomes anti-Hebbian and the probability of reproducing similar behavior in the future decreases (Dehaene and Changeux 1989, 1991, 1993, 1997; Dehaene, Kerszberg, and Changeux 1998). More complex rules have also been suggested (Sutton and Barto 1998).

The above-described reward-selection rule results in behavioral changes on a slow timescale. Because of small and widespread cumulative synaptic modifications, learning typically takes hundreds to thousands of trials, a mechanism compatible with the timescale of operant conditioning procedures in animals.

Anticipation of Reward

A faster reward-dependent learning is achieved through a more elaborate process referred to as auto-evaluation (Dehaene and Changeux 1991), value prediction (Friston et al. 1994; Sutton and Barto 1998), or reward expectation (Schultz et al. 1997), which anticipates future external rewards. The output of this reward-expectation system, rather than the actual external reward itself, is then used to direct behavioral changes. It accelerates learning and partially solves the credit-assignment problem since each action can be immediately associated with an increase or decrease in the probability of subsequent rewards (Sutton and Barto 1998). Most importantly, it gives the organism access to an internal mode of "mental simulation" in which various courses of action can be evaluated without taking risks in trying them out on the external world and wasting energy (Dehaene and Changeux 1991, 1997). Schultz and his collaborators (review in Schultz et al. 1997) have suggested that the expectation of rewards mobilizes a circuit involving the dopaminergic neurons in the VTA and SN and have further proposed to capture its features by a theoretical model of reinforcement learning referred to as the temporal-difference algorithm (Schultz et al. 1997; Sutton and Barto 1998). Yet, these views remain controversial at the physiological level (see, e.g., Pennartz 1995; Redgrave et al. 1999; Spanagel and Weiss 1999).

Selection of an "Adequate" Action

The elementary mechanism of selection by an auto-evaluated reward initially proposed (Dehaene and Changeux 1991; Dehaene, Naccache et al. 1998) was restricted to a set of clusters of prefrontal neurons encoding a repertoire of behavioral rules whose activation controls a lower-level sensorimotor network. Clusters were postulated to exhibit a high level of spontaneous activity together with strong recurrent connectivity and thus to display two stable modes of activity: one in which the cluster is inactive and the other in which activity remains at a high level for a prolonged period. Once activated, clusters remain in a state of self-sustained activation for a long duration.

Action selection is implemented by a stabilization–destabilization mechanism (Dehaene and Changeux 1989, 1991). Negative reinforcement is assumed to cause a fast synaptic desensitization with a timescale of a few tens of milliseconds; later, the synapses spontaneously recover their original strength with a slower timescale of a few seconds. The net result of this mechanism is that whenever negative reinforcement is received, recurrent connections within the currently active cluster rapidly decrease in strength, thus releasing the neighboring clusters from lateral inhibition. Spontaneous activity then wanders from one cluster to another, giving the organism the chance to test different prerepresentations and behavioral options; thus, reward signals function as effective selection signals that either maintain or suppress currently active prefrontal representations as a function of their current adequacy.

Relevant to these issues is the observation that patients with lesions in the prefrontal (in particular the orbitofrontal) cortex show inabilities to judge such adequacy, that is, to adapt thought and behavior to ongoing reality, a disorder known as spontaneous confabulation (Schnider 2003). Electrophysiological studies on memory suppression and extinction showed that prefrontal activity occurs very early after presentation of currently irrelevant or not anticipated stimuli, even before their content is actually consciously recognized (Schnider et al. 2002; Schnider 2003). This observation is consistent with a fast desensitization of negative reinforcement and, further, localizes this monitoring system in the orbitofrontal cortex.

At the molecular level, the reward signal is postulated to be a neurotransmitter such as dopamine, acetylcholine, or a coexisting messenger exerting a global modulatory action. Most models of synaptic modification are based on the coincidence-detection properties of the NMDA glutamate receptor, that is, on the direct effect of electrical fields on ion channel block by Mg^{2+} ions (Wigström and Gustafsson 1985). At variance, the tentative molecular mechanism suggested for how a reward signal is involved in decision selection relies upon the known properties of a large body of non-NMDA neurotransmitter receptor molecules, the archetype of which is the nicotinic acetylcholine receptor together with its allosteric transitions of activation and desensitization (review in Changeux and

Edelstein 1998, 2005). The transmembrane polarity of the receptor molecule indeed allows allosteric effectors, including electrical signals, second messengers (including phosphorylations) and "reward signals" to cooperate through the membrane in particular when their sites are located on the opposite faces. Convergence of their effects within a given time window may then result in a Hebbian regulation of postsynaptic receptor efficacy (Heidmann and Changeux 1982; Changeux and Edelstein 1998, 2005). Such a mechanism would differ from, and possibly complement, that proposed for the NMDA-type glutamate receptor. It provides potential means for interaction between signaling systems in the timescale of the activation/desensitization transitions of receptors (few ms to 100s of ms or even longer) and thus to the fast integration of neurons into larger assemblies (Lansner et al. 2002; Sandberg et al. 2003; Frégnac et al., this volume).

Lastly, these allosteric molecules are the target of many psychotropic drugs. This is, for instance, the case of benzodiazepines, which potentiate, in an allosteric manner, the GABA receptor and this may suffice to control the subjective state of anxiety in our brain. The dynamics of these elementary molecular switches may thus impose, in a bottom-up manner, insurmountable limits to the higher functions of the brain.

The global action of neuromodulatory reward messengers thus create an efficient—though still hypothetical—mechanism of global integration of the organism to its environment.

CONCLUSION

The aim of this brief review was to present and discuss plausible, but still largely hypothetical neural architectures and physiological processes, which, at the highest level, may contribute to the integration of microcircuits' (or ensemble of microcircuits') activities into brain-scale "workspace representations" possibly involved in mental syntheses and decision making. First, a processing network composed of parallel, distributed, and functionally encapsulated processors organized from brain microcircuits is distinguished from a global workspace assumed to consist of a distributed set of cortical neurons, which physically integrate, at the brain-scale level, the multiple processors by their long-range excitatory axons (Dehaene, Kerszberg, and Changeux 1998; Dehaene, Naccache et al. 1998; Dehaene et al. 2003). In parallel, "global" electrophysiological recordings (Lehmann 1990; Michel et al. 1999) reveal a striking segmentation of the EEG traces into discrete "functional microstates." The suggestion is made, which still deserves demonstration, that the "melody" of these microstates reflects the dynamics of the conscious content of the neuronal workspace. Both approaches further underline the top-down contribution of ongoing spontaneous activity to the all-or-none access of sensory stimuli to "consciousness." Last, in the final section, a model for reward-dependent selection of

representations (Dehaene and Changeux 2000) within the neuronal workspace was presented. It offers a plausible learning mechanism by selection, which might possibly contribute to the integration of the organism with itself but also with its environment.

One of the major challenges raised by these modeling attempts is to establish plausible causal links between the molecular and cognitive "conscious" levels, thus leading to specific behavioral and "mental" states predictions grounded in molecular and pharmacological mechanisms. For instance, the transitions between conscious states are under the control of global modulatory neural systems, among them cholinergic and noradrenergic (Stériade 2003; Llinas and Ribary 1998; Léna and Changeux 1997; Cohen et al. 2002; Léna et al. 2004): thus the plausible explanation for (a) the decrease of "microarousals" occurring during slow-wave sleep (Léna et al. 2004) in mice lacking nAChR $\alpha 4$ and/or $\beta 2$ subunit as well as for (b) the consequences of constitutive "gain of function" mutations known to alter the allosteric properties of neuronal nAChR (in the $\alpha 4$ and/or $\beta 2$ subunit) (Changeux and Edelstein 1998) and causing nocturnal frontal lobe epilepsies (Steinlein et al. 1995; Steinlein 2004) which crises occur during light slow-wave sleep precisely at the time where microarousals occur (Sutor and Zolles 2001).

Also, the neuronal workspace model (Dehaene and Changeux 2005) relies upon both a bottom-up neural network that propagates sensory stimulation across the hierarchy of areas and a long-distance and a top-down network that sends amplification signals back to all levels below it, yet with different neurochemical implementation. Among others, one may mention fast glutamatergic AMPA receptors for bottom-up propagation, and slower glutamatergic NMDA receptors for top-down amplification.

These plausible but still hypothetical implementations have important consequences in the understanding of the numerous pathologies affecting conscious states (autism and schizophrenia, among others), the development of therapeutic strategies against these diseases, and the chemistry of general anesthesia.

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REFERENCES

Arbib, M., P. Erdi, and J. Szentagothai. 1998. Neural Organisation: Structure, Function, and Dynamics. Cambridge, MA: MIT Press.

Baars, B.J. 1988. Cognitive Theory of Consciousness. Cambridge: Cambridge Univ. Press.
Bergson, H. 1959. Œuvres. Paris: PUF.
Braeutigam, S., and S.J. Swithenby. 2003. Endogenous context for visual processing of human faces and other objects. *NeuroReport* **14**:1385–1389.
Cajal, S.R. 1909. Histologie du système nerveux de l'homme et des vertébrés. Paris: Maloine.
Changeux, J.-P. 1983. L'homme neuronal. Paris: Fayard.
Changeux, J.-P. 2005a. Genes, brains, and culture: From monkey to human. In: From Monkey Brain to Human Brain, A Fyssen Foundation Symposium, pp. 73–94. Cambridge, MA: MIT Press.
Changeux, J.-P. 2005b. The Physiology of Truth. Cambridge, MA: Harvard Univ. Press.
Changeux, J.-P., and S.J. Edelstein. 1998. Allosteric receptors after 30 years. *Neuron* **21**:959–980.
Changeux, J.-P., and S.J. Edelstein. 2005. Nicotinic Acetylcholine Receptors. New York: Odile Jacob.
Churchland, P.S., and T.J. Sejnowski. 1992. The Computational Brain. Cambridge, MA: MIT Press.
Cohen, J.D., W.M. Perlstein, T.S. Braver et al. 1997. Temporal dynamics of brain activation during a working memory task. *Nature* **386**:604–608.
Cohen, L., S. Lehericy, F. Chochon et al. 2002. Language-specific tuning of visual cortex? Functional properties of the Visual Word Form Area. *Brain* **125**:1054–1069.
Crick, F. 1994. The Astonishing Hypothesis. New York: C. Scribner.
Crick, F., and C. Koch. 2003. A framework for consciousness. *Nat. Neurosci.* **6**:119–126. Review.
Dehaene, S., and J.-P. Changeux. 1989. A simple model of prefrontal cortex function in delayed-response tasks. *J. Cogn. Neurosci.* **1**:244–261.
Dehaene, S., and J.-P. Changeux. 1991. The Wisconsin card sorting test: Theoretical analysis and simulation of a reasoning task in a model neuronal network. *Cereb. Cortex* **1**:62–79.
Dehaene, S., and J.-P. Changeux. 1993. Development of elementary numerical abilities: A neuronal model. *J. Cogn. Neurosci.* **5**:390–407.
Dehaene, S., and J.-P. Changeux. 1997. A hierarchical neuronal network for planning behavior. *PNAS* **94**:13,293–13,298.
Dehaene, S., and J.-P. Changeux. 2000. Reward-dependent learning in neuronal networks for planning and decision making. *Prog. Brain Res.* **126**:217–229.
Dehaene, S., and J.-P. Changeux. 2004. Neural mechanisms for access to consciousness. In: The Cognitive Neurosciences III, ed. M. Gazzaniga, pp. 1145–1158. Cambridge, MA: MIT Press.
Dehaene, S., and J.-P. Changeux. 2005. Ongoing spontaneous activity controls access to consciousness: A neuronal model for inattentional blindness. *PLOS Biol*. **3**:e141.
Dehaene, S., J.-P. Changeux, and J.P. Nadal. 1987. Neural networks that learn temporal sequences by selection. *PNAS* **84**:2727–2731.
Dehaene, S., M. Kerszberg, and J.-P. Changeux. 1998. A neuronal model of a global workspace in effortful cognitive tasks. *PNAS* **95**:14,529–14,534.
Dehaene, S., and L. Naccache. 2002. Towards a cognitive neuroscience of consciousness: Basic evidence and a workspace framework. *Cognition* **79**:1–37.
Dehaene, S., L. Naccache, G. Le Clec'H et al. 1998. Imaging unconscious semantic priming. *Nature* **395**:597–600.

Dehaene, S., C. Sergent, and J.-P. Changeux. 2003. A neuronal network model linking subjective reports and objective physiological data during conscious perception. *PNAS* **100**:8520–8525.

Eccles, J. 1989. Evolution of the Brain: Creation of the Self. New York: Basic Books.

Edelman, G.M. 1987. Neural Darwinism. New York: Basic Books.

Efron, R. 1970. The minimum duration of a perception. *Neuropsychologia* **8**:57–63.

Engel, A.K., P. Fries, and W. Singer. 2001. Dynamic predictions: Oscillations and synchrony in top-down processing. *Nat. Rev. Neurosci*. **2**:704–716.

Everitt, B.J., A. Dickinson, and T.W. Robbins. 2001. The neuropsychological basis of addictive behaviour. *Brain Res. Brain Res. Rev*. **36**:129–138.

Faure, P., and H. Korn. 1997. A nonrandom dynamic component in the synaptic noise of a central neuron. *PNAS* **94**:6506–6511.

Fessard, A. 1954. Nervous Integration and Conscious Experience. Symposium Sainte-Marguerite. London: Blackwell.

Fransén, E., and A. Lansner. 1998. A model of cortical associative memory based on a horizontal network of connected columns. *Network: Comput. Neural Syst.* **9**:235–264.

Friston, K.J., G. Tononi, G.N. Reeke, Jr., O. Sporns, and G.M. Edelman. 1994. Value-dependent selection in the brain: Simulation in a synthetic neural model. *Neuroscience* **59**:229–243.

Gonzalez Andino, S., C.M. Michel, G. Thut, T. Landis, and R. Grave de Peralta. 2004. Prediction of response speed by anticipatory high-frequency (gamma band) oscillations in the human brain. *Human Brain Map*. **24**:50–58.

Hebb, D. 1949. The Organization of Behavior. New York: Wiley.

Heidmann, T., and J.-P. Changeux. 1982. Molecular model of the regulation of chemical synapse efficiency at the postsynaptic level. *C.R. Acad. Sci. III* **295**:665–670.

Hobson, J.A. 1999. Arrest of firing of aminergic neurones during REM sleep: Implications for dream theory. *Brain Res. Bull*. **50**:333–334.

Holt, E. 1931. Animal Drive and the Learning Process. New York: Henri Holt.

Hull, C. 1943. Principles of Behavior. New York: Appleton.

Jacobsen, C.F. 1935. Function of frontal association cortex in primates *Arch. Neurol. Psychiatry* **33**:558–560.

James, W. 1890. The Principles of Psychology. Cambridge, MA: Harvard Univ. Press.

John, E.R. 2002. The neurophysics of consciousness. *Brain Res. Rev.* **39**:1–28.

Jones, E. 1998. A new view of specific and nonspecific thalamocortical connections. In: Consciousness: At the Frontiers of Neuroscience, ed. H.H. Jasper, L. Descarries, V.F. Castellucci, and S. Rossignol, Advances in Neurology vol. 77, pp. 49–73. New York: Lippincott-Raven Press.

Kandel, E., J. Schwartz, and T. Jessel. 1995. Essentials of Neural Science and Behavior. Stamford, CN: Appleton and Lange.

Khateb, A., C.M. Michel, A.J. Pegna et al. 2003. Processing of semantic categorical and associative relations: An ERP mapping study. *Intl. J. Psychophysiol.* **49**:41–55.

Khateb, A., C.M. Michel, A.J. Pegna, T. Landis, and J.M. Annoni. 2000. New insights into the Stroop effect: A spatio-temporal analysis of electric brain activity. *NeuroReport* **11**:1849–1855.

Koch, C. 2004. The Quest for Consciousness. Englewood, CO: Roberts and Co.

Koenig, T., L. Prichep, D. Lehmann et al. 2002. Millisecond by millisecond, year by year: Normative EEG microstates and developmental stages. *Neuroimage* **16**:41–48.

Kondakor, I., D. Lehmann, C.M. Michel et al. 1997. Prestimulus EEG microstates influence visual event-related potential microstates in field maps with 47 channels. *J. Neural Transm.* **104**:161–173.

Kondakor, I., R.D. Pascual-Marqui, C.M. Michel, and D. Lehmann. 1995. Event-related potential map differences depend on the prestimulus microstates. *J. Med. Eng. Technol.* **19**:66–69.

Koukkou, M., and D. Lehmann. 1983. Dreaming: The functional state-shift hypothesis. A neuropsychophysiological model. *Br. J. Psychiatry* **142**:221–31.

Koukkou, M., and D. Lehmann. 1987. An information-processing perspective of psychophysiological measurements. *J. Psychophysiol.* 1:**109**–112.

Lansner, A., E. Fransén, and A. Sandberg. 2002. Cell assembly dynamics in detailed and abstract attractor models of cortical associative memory. *Theory Biosci.* **122**:19–36.

Lehmann, D. 1980. Fluctuations of functional state: EEG patterns, and perceptual and cognitive strategies. In: Functional States of the Brain: Their Determinants, ed. M. Koukkou, D. Lehmann, and J. Angst, pp. 189–202. Amsterdam: Elsevier.

Lehmann, D. 1990. Brain electric microstates and cognition: The atoms of thought. In: Machinery of the Mind, ed. E.R. John, pp. 209–224. Boston: Birkhäuser.

Lehmann, D. 1992. Brain electric fields and brain functional states. In: Evolution of Dynamical Structures in Complex Systems, ed. R. Friedrich and A. Wunderlin, pp. 235–248. Berlin: Springer.

Lehmann, D., P.L. Faber, S. Galderisi et al. 2005. EEG microstate duration and syntax in acute, medication-naïve, first-episode schizophrenia: A multi-center study. *Psychiatry Res. NeuroImaging* **138**:141–156.

Lehmann, D., K. Kochi, T. Koenig et al. 1995. Microstates of the brain electric field and momentary mind states. In: Quantitative and Topological EEG and MEG Analysis, ed. M. Eiselt, U. Zwiener, and H. Witte, pp. 139–146. Jena: Universitätsverlag Mayer.

Lehmann, D., C.M Michel, I. Pal, and R.D. Pascual-Marqui. 1994. Event-related potential maps depend on prestimulus brain electric microstate map. *Intl. J. Neurosci.* **74**:239–248.

Lehmann, D., W.K. Strik, B. Henggeler, T. Koenig, and M. Koukkou. 1998. Brain electric microstates and momentary conscious mind states as building blocks of spontaneous thinking: I. Visual imagery and abstract thoughts. *Intl. J. Psychophysiol.* **29**:1–11.

Léna, C., and J.-P. Changeux. 1997. Role of Ca^{2+} ions in nicotinic facilitation of GABA release in mouse thalamus. *J. Neurosci.* **17**:576–585.

Léna, C., D. Popa, R. Grailhe et al. 2004. β2-containing nicotinic receptors contribute to the organization of sleep and regulate putative micro-arousals in mice. *J. Neurosci.* **24**:5711–5718

Levin, E.D., and A.H. Rezvani. 2002. Nicotine treatment for cognitive dysfunction. *Curr. Drug Targets—CNS & Neurol. Disord*. **1**:423–431.

Libet, B. 1981. The experimental evidence of subjective referral of a sensory experience backward in time. *Philosophy Sci.* **48**:182–197.

Llinas, R., and U. Ribary. 1998. Temporal conjunction in thalamocortical transactions. *Adv. Neurol.* **77**:95–102.

Lumer, E.D., G.M. Edelman, and G. Tononi. 1997. Neural dynamics in a model of the thalamocortical system. II. The role of neural synchrony tested through perturbations of spike timing. *Cereb. Cortex* **7**:228–236.

McCarthy, R., and E.K. Warrington. 1990. Cognitive Neuropsychology: A Clinical Introduction. San Diego: Academic.

Michel, C.M., B. Henggeler, and D. Lehmann. 1992. 42-channel potential map series to visual contrast and stereo stimuli: Perceptual and cognitive event-related segments. *Intl. J. Psychophysiol*. **12**:133–145.

Michel, C.M., M. Seeck, and T. Landis. 1999. Spatiotemporal dynamics of human cognition. *News Physiol. Sci*. **14**:206–214.

Michel, C.M., G. Thut, S. Morand et al. 2001. Electric source imaging of human brain functions. *Brain Res. Rev.* **36**:108–118.

Miyashita, Y., and T. Hayashi. 2000. Neural representation of visual objects: Encoding and top-down activation. *Curr. Opin. Neurobiol*. **10**:187–194.

Mohr, C., C.M. Michel, G. Lantz et al. 2005. Brain state-dependent functional hemispheric disconnection in men but not women. *Cereb. Cortex* **15**:1451–1458.

Mountcastle, V. 1998. The Cerebral Cortex. Cambridge, MA: Harvard Univ. Press.

Murray, M.M., C.M. Michel, R. Grave de Peralta et al. 2004. Rapid discrimination of visual and multisensory memories revealed by electrical neuroimaging. *Neuroimage* **21**:125–135.

Pardo, J.V., P.J. Pardo, K.W. Janer, and M.E. Raichle. 1990. The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. *PNAS* **87**:256–259.

Pau, H. 2000. Last but not least. Positive scotomata of the blind spots during abrupt changes between darkness and twilight. *Perception* **29**:1499–1500.

Paus, T., L. Koski, Z. Caramanos, and C. Westbury. 1998. Regional differences in the effects of task difficulty and motor output on blood flow response in the human anterior cingulate cortex: A review of 107 PET activation studies. *NeuroReport* **9**:R37–47.

Pavlov, I. 1897. Lectures on the Work of the Digestive Glands. St. Petersburg: Kusheroff.

Pegna, A.J., A. Khateb, L. Spinelli et al. 1997. Unraveling the cerebral dynamics of mental imagery. *Human Brain Map.* **5**:410–421

Pennartz, C.M. 1995. The ascending neuromodulatory systems in learning by reinforcement: Comparing computational conjectures with experimental findings. *Brain Res. Brain Res. Rev.* **21**:219–245.

Poppel, E., R. Held, and D. Frost. 1973. Residual visual function after brain wounds involving the central visual pathways in man. *Nature* **243**:295–296.

Purpura, D.P. 1972. Discussion: Functional studies of thalamic internuclear interactions. *Brain Behav. Evol.* **6**:203–209.

Raichle, M.E., J.A. Fiez, T.O. Videen et al. 1994. Practice-related changes in human brain functional anatomy during nonmotor learning. *Cereb. Cortex* **4**:8–26.

Redgrave, P., T.J. Prescott, and K. Gurney. 1999. Is the short-latency dopamine response too short to signal reward error? *TINS* **22**:146–151.

Sacks, O. 2004. In the river of consciousness. *NY Rev. Books* **51**: January 15.

Sandberg, A., J. Tegnér, and A. Lansner. 2003. A working memory model based on fast Hebbian learning. *Network: Comput. Neural Syst.* **14**:789–802.

Schnider, A. 2003. Spontaneous confabulation and the adaptation of thought to ongoing reality. *Nat. Rev. Neurosci.* **4**:662–671.

Schnider, A., N. Valenza, S. Morand, and C.M. Michel. 2002. Early cortical distinction between memories that pertain to ongoing reality and memories that don't. *Cereb. Cortex* **12**:54–61.

Schultz, W., P. Dayan, and P.R. Montague. 1997. A neural substrate of prediction and reward. *Science* **275**:1593–1599. Review.

Searle, J.R. 2000. Consciousness. *Ann. Rev. Neurosci.* **23**:557–578.

Sergent, C., and S. Dehaene. 2004. Is consciousness a gradual phenomenon? Evidence for an all-or-none bifurcation during the attentional blink. *Psychol. Sci.* **15**:720–728.

Spanagel, R., and F. Weiss. 1999. The dopamine hypothesis of reward: Past and current status. *TINS* **22**:521–527.

Sporns, O., J.A. Gally, G.N. Reeke, Jr., and G.M. Edelman. 1989. Reentrant signaling among simulated neuronal groups leads to coherency in their oscillatory activity. *PNAS* **86**:7265–7269.

Sporns, O., G. Tononi, and G.M. Edelman. 1991. Modeling perceptual grouping and figure-ground segregation by means of active reentrant connections. *PNAS* **88**:129–133.

Steinlein, O.K. 2004. Nicotinic receptor mutations in human epilepsy. *Prog. Brain Res.* **145**:275–285.

Steinlein, O.K., J.C. Mulley, P. Propping et al. 1995. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat. Genet.* **11**:201–203.

Stériade, M. 2003. The corticothalamic system in sleep. *Front. Biosci.* **8**:d878–99.

Super, H., C. van der Togt, H. Spekreijse, and V.A. Lamme. 2003. Internal state of monkey primary visual cortex (V1) predicts figure-ground perception. *J. Neurosci.* **15**:3407–3414.

Sutor, B., and G. Zolles. 2001. Neuronal nicotinic acetylcholine receptors and autosomal dominant nocturnal frontal lobe epilepsy: A critical review. *Pflugers Arch.* **442**: 642–651.

Sutton, R., and A. Barto. 1998. Reinforcement Learning: An Introduction. Cambridge, MA: MIT Press.

Taylor, J.G. 1999. The Race for Consciousness. Cambridge, MA: MIT Press.

Thorndike, E. 1898. Animal intelligence: An experimental study of the associative process in animals. *Psychol. Rev.* **Suppl. 8**:1–109.

Tolman, E.C. 1951. Collected Papers in Psychology. Berkeley: Univ. of California Press.

Tononi, G., and G.M. Edelman. 2000. Schizophrenia and the mechanisms of conscious integration. *Brain Res. Brain Res. Rev.* **31**:391–400.

Tononi, G., O. Sporns, and G.M. Edelman. 1992. Reentry and the problem of integrating multiple cortical areas: Simulation of dynamic integration in the visual system. *Cereb. Cortex* **2**:310–335.

Tsodyks, M., T. Kenet, A. Grinvald, and A. Arieli. 1999. Linking spontaneous activity of single cortical neurons and the underlying functional architecture. *Science* **286**: 1943–1946.

Von Economo, C. 1929. The Cytoarchitectonics of the Human Cerebral Cortex. London: Oxford Univ. Press.

Wackermann, J. 1999. Towards a quantitative characterisation of functional states of the brain: From the non-linear methodology to the global linear description. *Intl. J. Psychophysiol.* **34**:65–80.

Wackermann, J., D. Lehmann, C.M. Michel, and W.K. Strik. 1993. Adaptive segmentation of spontaneous EEG map series into spatially defined microstates. *Intl. J. Psychophysiol.* **14**:269–283.

Weiskrantz, L. 1997. Fragments of memory. *Neuropsychologia* **35**:1051–1057.

Wigström, H., and B. Gustafsson. 1985. On long-lasting potentiation in the hippocampus: A proposed mechanism for its dependence on coincident pre- and postsynaptic activity. *Acta Physiol. Scand*. **123**:519–522.

Williamson, S.J., L. Kaufman, S. Curtis et al. 1996. Neural substrates of working memories are revealed magnetically by the local suppression of alpha. *J. Clin. Neurophysiol.* **16**:512–519.

18

Theory of the Computational Function of Microcircuit Dynamics

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ABSTRACT

This chapter discusses models for computation in cortical microcircuits in the light of general computational theories and biological data. We first review some basic concepts from computation theory. We then discuss existing models for computation in cortical microcircuits within this precise conceptual framework. We argue that models for online computing in dynamical systems require particular attention in this context, since they provide the best fit to the types of computational tasks that are solved by the brain, as well as the best fit to biological data about the anatomy and physiology of the underlying circuits.

INTRODUCTION

The neocortex has enabled a quantum leap in the ability of mammals to adapt to a rapidly changing environment by supporting the emergence of sophisticated cognitive functions. Higher cognitive function depends critically on the ability of a system to predict future events in order to generate appropriate and intelligent responses in a rapidly changing environment. The neocortex solves this computational challenge by transforming multisensory information in parallel and in real time into a multidimensional output using information that was processed and stored at almost any combination of time points in the past. Therefore, a central challenge is to understand how the neocortex is designed to solve this task and what kind of theoretical framework could explain the computational principles it uses to solve this task.

The neocortex is characterized by precise topographic maps where information from the senses is mapped onto different regions of the neocortex, and

different regions of the neocortex are mapped back onto subcortical brain regions and onto effector organs that drive the body. Different regions of the neocortex are also intricately mapped onto each other to fuse all the modalities into a coherent perception. These topographical maps onto, between, and from the neocortex specify precisely the primary function of each cortical region; they also mean that all functions of the neocortex are interlinked. It is the manner in which these functions are interlinked that forms the cognitive architectures that allow the neocortex to construct integrated high-dimensional sensorimotor models to simulate and predict future events.

The principle of precise topographic mapping combined with massive recurrent links is applied in the neocortex not only between brain areas, but down to the most detailed level of the circuit design. The neocortex is, for example, arranged into vertical layers, each with unique afferent and efferent connectivity; this allows different brain regions to map onto different layers of the same column of neocortex. The nonspecific thalamus is mapped onto layer I, association regions onto layers II and III, specific thalamus onto layer IV, higher association areas and specific thalamus are mapped onto layer V, and multiple brain regions as well as specific thalamic nuclei are mapped onto layer VI. In terms of output, layer II/III neurons provide the main output to association cortical regions, layer V provides the main output to subcortical regions and contralateral hemispheres, and layer VI provides the main output to thalamus and brain regions specializing in processing different modalities. While each layer is specialized to process primarily specific input and generate a specific output, the layers are also highly interconnected (Thomson and Morris 2002), indicating that information from multiple brain regions is interlinked in columns of neurons. It is the manner in which these layers are mapped onto each other that governs how sensory input, ongoing activity, and output are coordinated to create a coherent perception and appropriate response.

Within neocortical layers, neurons are also intricately mapped onto each other, where the anatomical and physiological properties as well as probabilities of connections between neurons are unique for each type of pre- and postsynaptic neuron combination (Gupta et al. 2000). The computational advantage of such a specific design of this recurrent microcircuit must be extremely powerful compared to a mere random recurrent network, because this intricate design is duplicated and applied throughout the neocortex in all mammalian species. Remarkable stereotypy exists in terms of morphology of cells (morphological stereotypy), electrical behavior of cells (electrophysiological stereotypy), positioning of cells (spatial stereotypy), patterning of the anatomical and physiological properties of synaptic connections between neighboring cells (local synaptic stereotypy), and in terms of long-range afferent and efferent connectivities (distal connection stereotypy) (Silberberg et al. 2002). Although there clearly exists a unique design with a considerable degree of stereotypy across different regions, ages, and species, variations are found that seem to be adaptations to the specific requirements of the brain region or species.

The stereotypy of the neocortical microcircuit led many early anatomists to propose that neocortical neurons could be grouped anatomically into columns, roughly the diameter of the spread of the basal axonal and dendritic arbors of pyramidal neurons (about 500 μm) (see Silberberg et al. 2002). Indeed, more than 80% of the synapses of neocortical interneurons are devoted to interconnecting neurons within a diameter of 500 μm. Experiments in the late 1950s and early 1960s further indicated that the neocortical sheet may also be regarded functionally as being composed of small repeating columns of several thousand cells of about 500 μm in diameter. Such functional modules have now been observed in many neocortical areas and in many species. More recent experiments have revealed multiple overlying functional columns, which indicate that the notion of a cortical column as a set anatomical entity is not correct. Functional modules are overlaid (Swindale et al. 2000) such that a group of neurons collaborating to perform one operation may not necessarily collaborate to perform a different operation. The dense local connectivity within a diameter of 500 μm, embedded in a continuous sheet of interconnected neurons, seems therefore to impart the neocortex with the remarkable ability of allowing multiple functional columns to form and overlie dynamically on the same cortical sheet. The properties of the local as well as afferent and efferent connectivity and the microcircuit continuity enable a functional neocortical column to form potentially at any point in the neocortex.

In summary, the neocortex is composed of heterogeneous microcircuits that differ with age, across brain regions, and across species. Nevertheless, many properties of the microcircuit are stereotypical, suggesting that neocortical microcircuits are merely variations of a common microcircuit template. Such a template could subserve the impressive computational capability of the neocortex, and diversification could allow microcircuits to meet the specific requirements demanded by different neocortical areas, environmental conditions, and species adaptations. The theoretical question is how such a generic template can display sufficiently powerful and versatile information-processing capabilities.

Below we introduce some basic concepts that are useful for a more rigorous discussion of this question in the context of computation theory. Thereafter we review a few existing hypotheses regarding the computational function of microcircuit dynamics in the light of various computational theories. Consequences for future research are discussed in the final section.

BASIC CONCEPTS FROM THE THEORY OF COMPUTING

We begin by defining a few basic concepts that are useful for highlighting characteristic differences among competing models for cortical computation. We refer to Savage (1998) for details regarding general computational models. Details on the computational power of artificial neural networks can be found in Sima and Orponen (2003). An elementary introduction to artificial neural networks is given in Tsodyks (2002).

Input–Output Conventions

If all of the input for a computation is available when the computation begins, one speaks of *batch input*. In contrast, *online computations* receive a possibly "never-ending" stream of inputs and need to be able to integrate information from previously received input segments with information from the current input segment. If the output of a computation has to be delivered by a specific deadline (e.g., within 100 ms after a specific input segment has arrived), one calls this *real-time computing*. If the computational machinery can even be prompted at any time to provide its current best guess of a suitable output, without a prespecified schedule for output demands as in real-time computing, then it implements an *anytime algorithm*. If there exists no deadline for delivering the output of a computation, this is referred to as *offline computing*[1].

Programs, Learning, and the Difference between Computing and Learning

The *program* of a computational model specifies which algorithm is applied to what type of input, and where and when the output of a computation is provided. One usually contrasts fully programmed computational models (such as Turing machines or cellular automata) with models that are able to learn (such as multilayer perceptrons with backprop). One usually says that a machine (or organism) *learns* when information contained in current inputs affects the way in which it processes future inputs. Of course, the way in which current inputs can change future processing has to be prespecified by a *learning algorithm*. Thus a computational device that learns also requires a program—one on a higher level that specifies the organization of learning for the system (i.e., how the handling of future inputs is affected by current or preceding inputs). Even "self-organizing" computational models require such higher-level programs (which might, of course, be encoded through distributed local operating rules).

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[1] Offline computing in combination with batch input is the most common mode considered in computational complexity theory, where upper bounds are provided for the number of computation steps that a specific algorithm needs on a specific computational model for completing its computation for any input of length n. Such upper bounds are usually expressed by terms that may, for example, have the form $c_1 \cdot n^d + c_2$ with arbitrary constants c_1, c_2 (abbreviated $O(n^d)$) that absorb all finite size effects into the constants c_1 and c_2. In the more general parts of computational complexity theory, one focuses on the exponent d. For example, the best-known complexity class consists of all families of computational problems for which there exists a (deterministic) algorithm and some finite exponent d so that any instance of this computational problem of any length n can be solved on a Turing machine within time $O(n^d)$ by this algorithm. Although this complexity class P is frequently viewed as a characterization of the universe of computationally solvable problems, one has to be careful in applications of these asymptotic concepts to scenarios such as computations in the brain, where it is clear that only inputs of length $n \leq n_0$ (for some specific finite bound n_0) can occur, and finite size effects that determine, for example, the size of the previously mentioned constants c_1 and c_2 may become quite relevant. It should also be mentioned that the class P, as well as most other concepts from classical computational complexity theory, are only meaningful for offline computations on batch input.

The preceding definitions are only meaningful for computations on batch inputs. The analysis of learning machines (or organisms that learn) becomes conceptually more difficult when one looks at online computing, since there one cannot talk about "current" and "future" inputs, one just has a (virtually) endless input stream. One could, of course, talk about current and future segments of this input stream, but even very simple devices for online computing, such as linear filters or finite automata, have the property that current input segments will influence the way in which future input segments will be processed, without any "learning" or "adaptation" being involved. Phrased differently, for any model of online computing that integrates information from several preceding input segments for its current output (i.e., for any model that is capable of *temporal integration* of information), it becomes difficult or even impossible to distinguish computing conceptually from learning. The "difference" becomes usually a matter of perspective, where, for example, online computations that take place on larger timescales, or which involve higher levels of computational organization, might be referred to as learning.

Internal States and Transitions between States

The internal state of a computational model (or of an autonomously learning system) at time t should contain all information that would have to be stored if one interrupts its processing at time t for a while and then wants to restart it and continue its operation at a later time $t + \Delta$ based on the information contained in this stored information, as if there had been no interruption. Such internal states can be digital or analog and can form finite or infinitely large sets of (possible) internal states. A finite automaton (or finite state machine) is characterized by the fact that it only has a finite state set, whereas a Turing machine (which is basically just a finite automaton together with a read/write tape) has an infinite set of digital states, since its current tape inscription (whose length is finite at any given time t, but may grow with t) is also part of its current internal state.

Transitions between internal states can be deterministic or stochastic. In the deterministic case, a program determines transitions between internal states (dependent on the current state and current input). In the case of stochastic computations, a program specifies the probability that a particular state s is assumed at time $t + 1$, given the state assumed at time t and the current input.

For computational models that work in continuous time, one commonly writes the program in the form of a differential equation. The resulting computational models are referred to as *dynamical systems*.

Computational Goals

Computational goals may be very specific, for example, to multiply two numbers or, more general, such as predicting future inputs or surviving for a long

time in a complex real environment with limited resources. Very specific computational goals, as in the first example, are characteristic of our current use of digital computers, whereas more general computational goals are potentially more characteristic of the brain.

Examples of Computational Models

Turing machines are computational models that are particularly suited for studying deterministic offline computations on digital batch input in discrete time with a number of internal states, which is finite at any time t, but may grow unboundedly with t. Turing machines are *universal* in the sense that one has not been able to come up with any digital computation that cannot be carried out by a Turing machine.

Digital or analog feedforward circuits (e.g., feedforward Boolean circuits or feedforward neural nets) constitute another class of standard models for offline computing. They can also be used for real-time computing (in the form of pipelining) since their computation time on any (batch) input is limited by the depth of the circuit. However, they can only be used for those computations on sequences of inputs where no temporal integration of information from several successively presented batch inputs is required.

Finite automata are computational models with a fixed finite set of internal states that are suitable for online computations in discrete time; in fact, they are perfectly suited for real-time computing. Their current state can hold information about current and past inputs, and their state transitions can be deterministic or stochastic. Cellular automata are ensembles of infinitely many identical copies of some finite automaton located on the nodes of some infinite graph, where every node has the same finite number of neighbors (e.g., a two-dimensional grid). At every discrete time step, each automaton changes its state and determines the output to its direct neighbors dependent on the inputs that it has received from its direct neighbors at the end of the preceding time step.[2] The input to a cellular automaton is commonly encoded in the initial states of the individual finite automata. It is well known that every Turing machine (hence any currently existing digital computer) can be simulated by some cellular automaton.[3]

Artificial neural networks are also usually considered only with discrete time, but with analog internal states (so that even a single internal state may contain infinitely many bits of information). Both deterministic and stochastic state transitions are considered. Feedforward neural nets are primarily used for

2 The well-known "Game of Life" is an example of such a cellular automaton.

3 To be precise, this holds only for a cellular automaton consisting of infinitely many cells. A cellular automaton consisting of just finitely many cells can only simulate those Turing machine computations that can be carried out within a corresponding space bound. Basic results from computational complexity theory imply that the computational power of Turing machines (and hence also of cellular automata) does not saturate at any finite space bound.

offline computing since they cannot integrate information from successive inputs; however, recurrent neural nets are suitable for offline and online computations. Although the learning capability of artificial neural networks is viewed as one of their essential properties, the organization of learning for neural networks is usually not controlled by the network itself, but by an external supervisor.[4] From that perspective neural network models are far away from being autonomously learning systems.

Genetic (or evolutionary) algorithms are programs for computational models with stochastic state transitions in discrete time whose computational goal is the generation of formal objects ("agents") that have high "fitness" according to a fitness function that is part of the program.

OPTIONS FOR UNDERSTANDING THE COMPUTATIONAL FUNCTION OF MICROCIRCUIT DYNAMICS

Obviously the computational function of the brain is to enable an autonomous system to survive in the real world. Often the computational function of the brain is seen more narrowly, conceptually separating computing from learning. However, as discussed in the preceding section, such distinction is not very meaningful for analyzing online computations on input streams (only for isolated computations on batch inputs).[5] Hence one needs to understand learning as an integral part of the computational function of neural microcircuits.

In the subsequent subsections, we will review three categories of computational models for neural microcircuits that differ with regard to the type of computation that is supported by these models (offline vs. online computing) and with regard to the circuit structures on which they focus (feedforward vs. recurrent circuits). In addition, the models also differ with regard to their underlying assumption about (a) the computational specialization of cortical microcircuits (Do cortical microcircuits exist that carry out just one particular computation?), and (b) how the computational function is "programmed" into cortical microcircuits (Is their computational function directly genetically programmed or acquired by learning in the course of genetically programmed developmental procedures and fine-tuned by synaptic plasticity throughout adulthood?).

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4 For example, the selection of training examples of a learning rate or of the time points when learning is turned on and off are typically taken over by a human supervisor, and are not carried out by the "learning device" (e.g., a multilayer perceptron) itself. In that sense, a multilayer perceptron with backprop cannot be viewed as an autonomously learning machine.

5 Separate trials in neurophysiological experiments often aim at testing an organism on artificially created batch inputs and proceed on the assumption that one can ignore the fact that from the perspective of the organism they still constitute segments of one continuous input stream.

Microcircuits as Modules That Compute Stereotypical Basis Functions for Computations on Batch Input

One can compute any Boolean function (i.e., any function f: $\{0,1\}^m \rightarrow \{0,1\}^n$ for arbitrary $m, n \in \mathbf{N}$)[6] on a feedforward circuit consisting of units or subcircuits that compute certain stereotypical *basis functions*. For example, it suffices to have subcircuits that compute an OR of two input bits in conjunction with subcircuits that compute a negation. It even suffices to iterate stereotypical copies of a single subcircuit, for example, of a subcircuit that computes (x_i AND NOT x_2). From a mathematical point of view there is nothing special about such basis functions, and many different sets of basis functions exist that are complete in the sense that all Boolean functions can be generated by them.

For analog computations it is more meaningful to look at ways of approximating (rather than computing) arbitrary given functions f: $[-B, B]^m \rightarrow [-B, B]^n$ from real numbers into real numbers by circuits that are composed of stereotypical subcircuits that compute suitable basis functions. Again there exist many different sets of basis functions that are complete in the sense that any continuous function f: $[-B, B]^m \rightarrow [-B, B]^n$ can be approximated arbitrarily closely[7] through suitable combinations of such basis functions. Since continuous functions can be approximated arbitrarily closely by polynomials (on any bounded domain $[-B, B]^m$), it suffices, for example, to choose addition and multiplication of real numbers (in combination with real or rational constants) as basis functions. The universal approximation theorem from artificial neural networks states that one can also choose as basis functions sigmoidal gates applied to weighted sums of the inputs, that is, functions of the form:

$$\sigma\left(\sum_{i=1}^{k} w_i x_i\right) \text{ with } \sigma(x) = \frac{1}{1+e^{-x}}.$$

In fact, one can use here instead of the sigmoidal function σ almost any nonlinear function $h = \mathbf{R} \rightarrow \mathbf{R}$. As an alternative it suffices to use a single application of a winner-take-all-like nonlinearity in combination with subcircuits that compute just linear weighted sums (Maass 2000). Thus we see that also for the computation of analog functions there exist many different sets of basis functions that are complete.

A tempting hypothesis regarding the computational role of cortical microcircuits is that there exist genetically programmed stereotypical microcircuits that compute certain basis functions. Numerous ways in which circuits of

6 $\{0, 1\}^m$ is the set of all bit strings of length m. $[-B, B]$ is the set of all real numbers with absolute value bounded by B.

7 This means that for any given ε there exist such approximating function C so that $\|f(\underline{x}) - C(\underline{x})\| \leq \varepsilon$ for any $\underline{x} \in [-B, B]^m$, where $\|f(\underline{x}) - C(\underline{x})\|$ denotes the Euclidean distance between the points $f(\underline{x})$ and $C(\underline{x})$ in the n-dimensional space $\mathbf{R}^n$.

neurons can potentially compute a complete set of Boolean basis functions have been proposed (see, e.g., Shepherd and Koch 1998). For example, a single shunting synapse can in principle compute the Boolean function (x_i AND NOT x_2), which forms a complete basis. Also many possible ways in which single neurons or circuits of neurons can potentially compute basis functions for *analog* computing (e.g., addition and multiplication) have been collected in Table 1.2 of Shepherd and Koch (1998), which is reproduced in this chapter (see Table 18.1), and in Chapter 21 of Koch (1999).

A possible way in which circuits of neurons could implement a sigmoidal gate has been proposed in Maass (1997). In Pouget and Sejnowski (1997) products of a Gaussian function (e.g., of the retinal location of a cue) and a sigmoidal function (e.g., of eye position) are proposed as basis functions for sensorimotor transformations. They prove that these basis functions are complete in the sense

Table 18.1 Potential computational functions of various biophysical mechanisms in neural circuits (from Shepherd 1990; reprinted with permission of Oxford University Press).

Biophysical Mechanism	Neuronal Operation	Example of Computation	Timescale
Action potential initiation	Threshold, one-bit analog-to-digital converter		0.5–5 ms
Action potentials in dendritic spines	Binary OR, AND, AND–NOT gate		0.1–5 ms
Nonlinear interaction between excitatory and inhibitory synapses	Analog AND–NOT veto operation	Retinal directional selectivity	2–20 ms
Spine–triadic synaptic circuit	Temporal differentiation high-pass filter	Contrast gain control in the LGN	1–5 ms
Reciprocal synapses	Negative feedback	Lateral inhibition in olfactory bulb	1–5 ms
Low, threshold calcium current (I_T)	Triggers oscillations	Gating of sensory information in thalamic cells	5–15 Hz
NMDA receptor	AND–NOT gate	Associative LTP	0.1–0.5 s
Transient potassium current (I_A)	Temporal delay	Escape reflex circuit in *Tritonia*	10–400 ms
Regulation of potassium currents (I_M, I_{AHP}) via neurotransmitter	Gain control	Spike frequency accommodation in sympathetic ganglion and hippocampal pyramidal cells	0.1–2 s
Long-distance action of neurotransmitters	Routing and addressing of information		1–100 s
Dendritic spines	Postsynaptic modification of functional connectivity	Memory storage	∞

that weighted sums of such functions can approximate any desired continuous sensorimotor transformation. It is argued that the outputs of some parietal neurons can be approximated quite well by such basis functions. However, it is not known exactly how the computation of these basis functions is neurally implemented. A more general perspective of this basis function approach is given in Salinas and Sejnowski (2001).

Most approaches based on static basis functions do not provide good models for the biologically more realistic case of online computing on time-varying input streams. Furthermore, there are no good models for explaining how the composition of basis functions to larger computational modules is organized or learned by neural systems.

Microcircuits as Dynamical Systems Whose Input Is Encoded in the Initial State

Complementary approaches towards understanding the role of stereotypical cortical microcircuits for cortical computation emphasize that these microcircuits are synaptically connected in a highly recurrent manner, not in the way of a feedforward circuit. Thus, we will now focus on recurrent circuits. In this subsection, however, we discuss only approaches that focus on offline computations, that is, computations on batch input that are encoded in the initial state of the system. Turing machines fall into this category, but also cellular automata, recurrent neural networks (e.g., Hopfield nets, attractor neural networks), and other more general dynamical systems are traditionally considered in this offline computational mode.[8] In fact it appears that the majority of computational models currently considered in computational neuroscience fall into this category.

Recurrent circuits can be composed from the same basis functions as feedforward circuits (see previous section). However, a new problem arises. In a feedforward circuit, the computational control that determines which subcircuit is activated at any given moment is implicitly encoded by the underlying wiring diagram, which is in that case a directed acyclic graph. Computations in recurrent circuits that implement Turing machines, cellular automata, or recurrent neural nets in discrete time require a central clock in conjunction with a protocol that determines which subcircuit is active at which clock tick. Although such computational organization is theoretically also possible for a circuit consisting of integrate-and-fire neurons (Maass 1996), the biologically more realistic case is obviously that of computing in continuous time with the interaction between computing elements described by differential equations. In this way one arrives at special cases of dynamical systems. The (batch) input is traditionally encoded in the initial state of the dynamical system, and one usually waits until it has converged to an attractor, which could be a fixed point, a limit cycle, or a "strange

8 The latter models can also be used for ting; such less traditional uses will be discussed in the next subsection.

attractor" as considered in chaos theory. Even dynamical systems that are composed of very simple dynamic units (e.g., sigmoidal gates) can have very complicated dynamics, and it is not clear what particular contribution should be expected from cortical microcircuits if they are viewed as implementations of such dynamic units. Freeman (1975) proposed using a conceptual framework by Ketchalsky for classifying components of recurrent circuits (K0, KI, KII sets, etc.), but the nature of nonlinear recurrent circuits tends to be in the way of any meaningful decomposition into simpler components.

Synfire chains (Abeles 1991) have been proposed as potential dynamic modules of complex recurrent circuits that inject a particular timing structure into the recurrent circuit. This is theoretically possible since a synfire chain (in its most basic form) is a feedforward circuit whose subsets of neurons are activated in a sequential manner. However, no significant computations can be carried out by a single synfire chain. It has been conjectured that interactions between several overlapping synfire chains may attain significant computational power, but this has not yet been demonstrated (without postulating an "intelligent" higher-order structure). Obviously, one needs to find principles by which a network of synfire chains could be autonomously created, structured, and updated. Another interesting open problem is how such a network could learn to process *time-varying* input streams.

Microcircuits as Generic Modules for Online Computing in Dynamical Systems

We now consider models for the computational function of cortical microcircuits that allow them to carry out online computations on complex input streams (which requires temporal integration of information). Finite automata are capable of carrying out such computations, although only on digital inputs in discrete time. To implement an arbitrary finite automaton, it suffices to combine a feedforward Boolean circuit that computes the transition function between states with computational units that act as registers for storing and retrieving information. One possible neural implementation of such registers was proposed in Douglas et al. (1995), using hysteretic effects in recurrent circuits. Altogether the main weakness of finite automata as a conceptual framework for neural computation is their strongly digital flavor (discrete time and discrete states), which makes learning or adaptation (that in neural systems usually involves gradient descent in one form or another, except for genetic algorithms) less powerful in this context.

Another attractive conceptual framework for the analysis of neural computation on online input streams is provided by linear and nonlinear filters. Numerous biophysical mechanisms that implement specific linear and nonlinear filters have been identified (see Table 18.1; Shepherd and Koch 1998; Koch 1999). Marmarelis and Marmarelis (1978) introduced into neurophysiology a number

of useful techniques for modeling the input–output behavior of black-box neural circuits by linear and nonlinear filters. A more recent account is given in Rieke et al. (1997). These techniques rely on Volterra series (or equivalently Wiener series) as mathematical frameworks for modeling online computations of neural systems on continuous input streams or spike trains as inputs. Volterra series model the output of a system at time t by a finite or infinite sum of terms of the form:

$$\int_0^\infty \ldots \int_0^\infty h_d(\tau_1,\ldots,\tau_2)\cdot u(t-\tau_1)\cdot\ldots\cdot u(t-\tau_d)\,d\tau_1\ldots d\tau_d\,,$$

where some integral kernel h_d is applied to products of degree d of the input stream $u(\cdot)$ at various time points $t-\tau_i$ back in the past. Usually only linear ($d=1$) or quadratic ($d=2$) filters are used for modeling specific neural systems, since too many data would be needed to fit higher-order kernel functions h_d. Not all possible computations on input streams $u(\cdot)$ can be modeled by Volterra series (of any degree), since any Volterra series (with convergent integral terms) has automatically a fading memory, where features of the input streams $u(\cdot)$ at any specific time point in the past have decreasing influence on the current output at time t when t grows. In addition, a Volterra series can only model outputs that depend in a smooth manner on the input stream $u(\cdot)$. Thus they can, for example, model spike output only in the form of smoothly varying firing rates or firing probabilities. On the other hand this mathematical framework imposes no constraint on how slowly the memory fades and how fast the smoothly varying output changes its value.

This conceptual framework of filters, which has traditionally been used primarily for analyzing signal processing rather than computing, was recently used by Maass and Sontag (2000) and Maass et al. (2002, 2004) as the basis for a new approach towards understanding the computational function of microcircuit dynamics. The liquid state machine was introduced as a generalization of the model of a finite automaton to continuous time and continuous ("liquid") states (see Figure 18.1).

To make this model better accessible to learning than the finite automaton, it was postulated that the liquid states (i.e., that part of the current state of the circuit that is expressed in its spiking activity and, therefore, "visible" for readout neurons) generated by a neural microcircuit should contain sufficient information about the recent input stream $u(\cdot)$ that other neurons ("readout neurons") have to learn in order to *select* and *recombine* those parts of the information stream contained in the time-varying liquid state useful for their specific computational task. Here, it is natural to apply a variant of the basis function idea discussed earlier and look for possible characterizations of sets of *basis filters* (that could be implemented, for example, by specific components of cortical microcircuits), which endow the resulting liquid states with the capacity to absorb

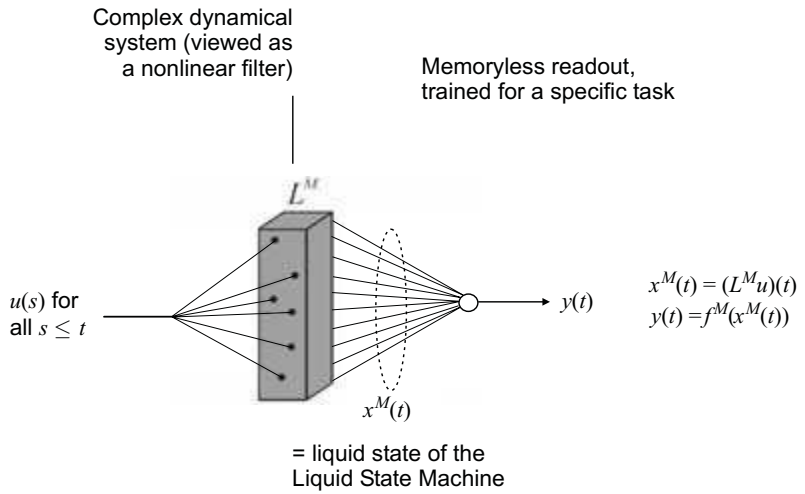
Complex dynamical system (viewed as a nonlinear filter)
Memoryless readout, trained for a specific task
L^M
$u(s)$ for all $s \leq t$
$y(t)$
$x^M(t) = (L^M u)(t)$
$y(t) = f^M(x^M(t))$
$x^M(t)$
= liquid state of the Liquid State Machine

Figure 18.1 Structure of a liquid state machine, which transforms input streams $u(\cdot)$ into output streams $y(\cdot)$.

enough information about the input stream $u(\cdot)$. A mathematical theorem (Maass et al. 2002) guarantees that sufficient *diversity* of the basis filters implemented by the components of a neural microcircuit, for example, neurons or dynamic synapses with sufficiently diverse time constants (Maass and Sontag 2000), suffices to endow the resulting liquid state machine with the capability to approximate in principle any input–output behavior that could potentially be approximated by a (finite or infinite) Volterra series. The available amount of diversity in a microcircuit can be measured indirectly via its separation property (Maass et al. 2002).

Another potential computational function of microcircuit dynamics arises if one considers such a liquid state machine from the perspective of an (approximately) linear readout neuron, for which it should become feasible to *learn* to select and recombine those aspects of liquid states that may be needed for specific tasks (e.g., smooth eye pursuit, or classification and prediction of dynamic visual scenes). A microcircuit can boost the capability of any linear readout by adding a certain redundancy to the information contained in its stream of liquid states, for example, also precomputing nonlinear combinations of salient time-varying variables (analogously as in the special case of gain fields), see Figure 18.2. Very recently, in Maass, Legenstein et al. (2005), a general quantitative method was developed to evaluate such *kernel capability* of neural microcircuits (where the notion of a *kernel* is used here in the sense of support vector machines in machine learning—it goes back to the idea of a fixed nonlinear preprocessing proposed already in the 1950s by Rosenblatt's work on perceptrons, but applied here to a time-varying context). Forthcoming new results show that the computational power of the resulting computational model becomes

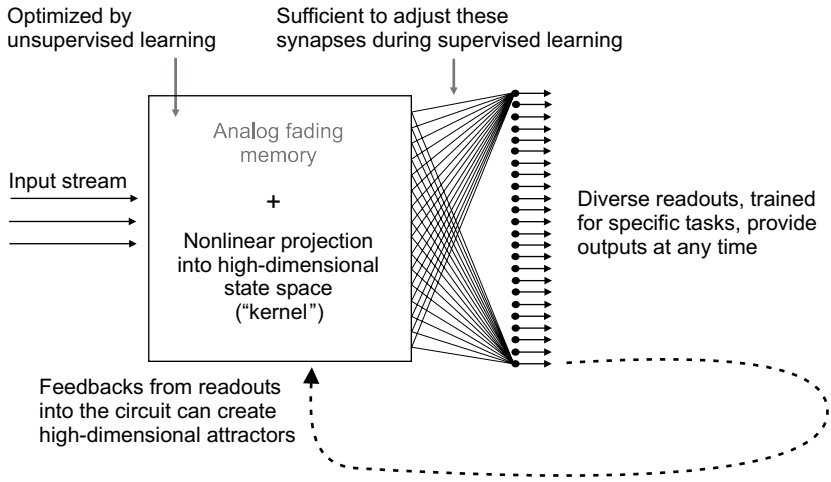

Figure 18.2 A system-oriented interpretation of the computational function of microcircuit dynamics.

significantly larger if feedback from trained readouts back into the circuit is also taken into account, as indicated by the dashed line in Figure 18.2 (Maass, Joshi et al. 2005).

From this perspective a cortical microcircuit is not viewed as an implementation of a single computation, but as a more universal computational device that can support simultaneously a large number of different computations. An example is given in Figure 18.3, where 7 different linear readouts from a generic neural microcircuit model consisting of 270 neurons had been trained to output at any time the result of 7 different computational operations on information provided to the circuit in the form of 4 spike trains (a sample is shown at the top of Figure 18.3). After training the weights of these linear readouts had been fixed. The results shown in Figure 18.3 are for new input spike trains that had never before been injected into the circuit, thereby demonstrating good generalization capability of this simple learning scheme (see Maass et al. 2002 for details).

Several experimental studies in the group of Yves Frégnac have shown that neurons can in fact be trained via current injections (even in adult animals *in vivo*) to read out particular aspects of the "liquid state" represented by the current firing activity of presynaptic neurons (see, e.g., Debanne et al. 1998). However, it has remained open by which principles readout neurons can be trained autonomously within a neural system to perform such task. This is least dubious in the case of prediction learning, where the arrival of the next input could provide such current injection into a readout neuron that learns to perform such prediction task as in the experiments of Debanne et al. (1998). These experimental data can be explained on the basis of standard rules for spike-timing-dependent plasticity (STDP) (see Legenstein et al. 2005). Another quite realistic scenario is

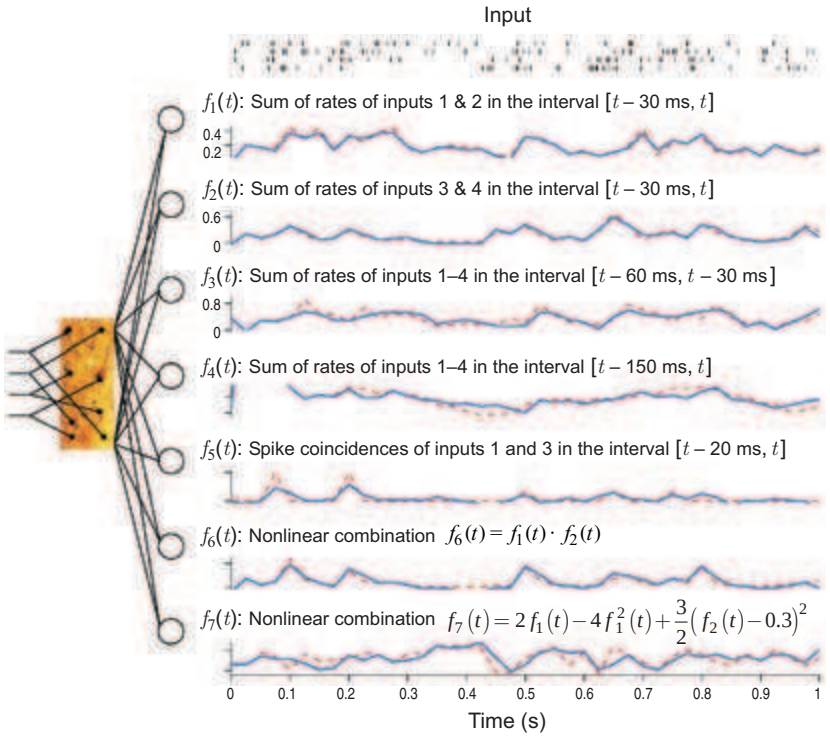
Input
$f_1(t)$: Sum of rates of inputs 1 & 2 in the interval $[t - 30$ ms, $t]$
0.4
0.2
$f_2(t)$: Sum of rates of inputs 3 & 4 in the interval $[t - 30$ ms, $t]$
0.6
0
$f_3(t)$: Sum of rates of inputs 1–4 in the interval $[t - 60$ ms, $t - 30$ ms$]$
0.8
0
$f_4(t)$: Sum of rates of inputs 1–4 in the interval $[t - 150$ ms, $t]$
$f_5(t)$: Spike coincidences of inputs 1 and 3 in the interval $[t - 20$ ms, $t]$
$f_6(t)$: Nonlinear combination $f_6(t) = f_1(t) \cdot f_2(t)$
$f_7(t)$: Nonlinear combination $f_7(t) = 2 f_1(t) - 4 f_1^2(t) + \frac{3}{2}\left(f_2(t) - 0.3\right)^2$
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Time (s)

Figure 18.3 Multi-tasking in real time. Input spike trains were randomly generated in such a way that at any time t the input contained no information about input rates that were used more than 30 ms ago. Firing rates $r(t)$ were randomly drawn from the uniform distribution over (0 Hz, 80 Hz) every 30 ms, and input spike trains 1 and 2 were generated for the present 30 ms time segment as independent Poisson spike trains with this firing rate $r(t)$. This process was repeated (with independent drawings of $r(t)$ and Poisson spike trains) for each 30 ms time segment. Spike trains 3 and 4 were generated in the same way, but with independent drawings of another firing rate $\tilde{r}(t)$ every 30 ms. The results shown in this figure are for test data that were never before shown to the circuit. Below the 4 input spike trains the target (dashed curves) and actual outputs (solid curves) of 7 linear readout neurons are shown in real-time (on the same time axis). Targets were to output every 30 ms the actual firing rate (rates are normalized to a maximum rate of 80 Hz) of spike trains 1 and 2 during the preceding 30 ms (f_1), the firing rate of spike trains 3 and 4 (f_2), the sum of f_1 and f_2 in an earlier time interval ($t - 60$ ms, $t - 30$ ms) (f_3) and during the interval ($t - 150$ ms, t) (f_4), spike coincidences between inputs 1 and 3 ($f_5(t)$ is defined as the number of spikes that are accompanied by a spike in the other spike train within 5 ms during the interval ($t - 20$ ms, t)), a simple nonlinear combination f_6, and a randomly chosen complex nonlinear combination f_7 of earlier described values. Since all readouts were linear units, these nonlinear combinations are computed implicitly within the generic microcircuit model. Average correlation coefficients between targets and outputs for 200 test inputs of length 1 s for f_1 to f_7 were 0.91, 0.92, 0.79, 0.75, 0.68, 0.87, and 0.65.

the case of association learning, where a "teaching current" could be injected into a readout neuron by projection neurons from other microcircuits in cortical or subcortical structure that are involved in processing inputs from other sensory modalities, or which signal external or internal rewards.

An attractive feature of this computational framework is that it produces a possible explanation for the *anytime computing capabilities* of neural systems, since readouts can learn to transform, at any moment in time, the currently provided liquid state into the best guess for a decision or a parameter value that is needed for their specific task. Another interesting aspect is that this approach is compatible with biological data regarding oscillations that are superimposed on sensory inputs (Kaske and Maass 2005). Häusler and Maass (2005) have also shown that this approach can be applied to more detailed cortical microcircuit models with data-based connectivity between cortical layers.

This approach also provides a computational explanation for the large-scale architecture of the brain, where sensory inputs and internal outputs are not spread out uniformly all over the brain (see INTRODUCTION). Rather, each brain area is characterized by the specific set of information streams that it receives. This feature is a prerequisite for online computing with dynamical systems, since different input streams that converge onto a single microcircuit all have an influence on its internal dynamics, thereby facilitating computations that depend on segments of all these information streams. Some other input stream that is not relevant for these computations would influence the microcircuit dynamics as well, but would represent a huge source of noise from the perspective of these computations, in particular blowing up and possibly interleaving the classes of equivalent circuit states (see Maass et al. 2004, Section 5) that a readout neuron has to learn to distinguish.

An essentially equivalent computational framework to liquid computing, echo state networks, has been developed independently in an engineering context (Jäger 2002), and currently surpasses all other known methods for various time series predition and adaptive nonlinear filtering tasks (Jäger and Haas 2004). Other recent work relates these approaches to earlier work by Chris Langton et al. on computation on the edge of chaos in dynamical systems (for a review, see Legenstein and Maass 2005a, b), but applied now to anytime computing on continuous input streams rather than to offline computations on static batch input (Bertschinger and Natschläger 2004). It is argued there that neither the "ordered" nor the "chaotic" regime of recurrent circuits (where recurrent circuits are viewed here as special cases of dynamical systems) are well suited for computing, but rather the regime in between (the "edge of chaos"). This has been demonstrated by Bertschinger and Natschläger (2004) for synchronized recurrent circuits consisting of threshold gates. Results of Maass, Legenstein et al. (2005) suggest that for more realistic models of neural microcircuits the "edge of chaos" becomes harder to conceptualize, and they propose measuring the computational power and generalization capability of neural microcircuits

instead by a quantitative measure of its kernel quality and a direct estimate of its generalization capability (VC-dimension).

Other Approaches

Various other approaches exist for explaining or modeling biological neural computation, which have not yet given rise to specific hypotheses regarding the computational role of cortical microcircuits. A number of interesting brain theories are based on information theoretic concepts, such as redundancy reduction, the information bottleneck method, and theories of optimal neural coding. Another family of models for biological neural computation uses generative models, which are based on the hypothesis that the large-scale computational goal of cortical computation is to reconstruct sensory inputs, and to "explain" them as being generated by independent components or factors that can be learnt in an unsupervised manner from the statistics of the inputs. An attractive feature of this approach is that it gives rise to autonomously learning systems. However, in a concrete biological context it is hard to demonstrate or even make precise the theoretically very attractive goal of reconstructing the input in terms of internally represented "hidden" sources. For example, top-down connections to primary sensory cortices appear to contribute to purpose-related interpretations of raw sensory input, rather than to the reconstruction of a (usually dynamically varying) sensory input; see, for example, Chapter 45 of Chalupa and Werner (2004) for the case of visual input. Unfortunately, the biologically more realistic goal of predicting future inputs (rather than reconstructing preceding inputs) has not yet given rise to an equally attractive theoretical approach.

DISCUSSION

This short survey shows that there exist drastic differences regarding theories on the computational function of cortical microcircuits. To find the most appropriate computational theory for a neural microcircuit one first needs to decide whether this circuit is designed to carry out offline computations on batch inputs or online computations on input streams. One also needs to decide to what extent this circuit is genetically programmed to carry out one specific computational operation, or whether it is designed to provide numerous and diverse "neural users" with a suitably processed amalgamation of the input streams that enter this microcircuit, where the specific output delivered to any particular "neural user" is to some degree shaped by learning.

The computational function of various salient aspects of cortical microcircuits is presently still not known, especially the computational role of specific cortical layers with particular types of neurons that are connected by particular types of synapses with particular probabilities with other specific types of

neurons on specific layers. Another big open problem is the organization of learning in cortical microcircuits; for example, we need to know how the plasticity of its synapses is gated, and what other processes regulate the computational function of its neurons in dependence of their individual history and the statistics of their inputs.

ACKNOWLEDGMENTS

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REFERENCES

Abeles, M. 1991. Corticonics: Neural Circuits of the Cerebral Cortex. Cambridge: Cambridge Univ. Press.

Bertschinger, N., and T. Natschläger. 2004. Real-time computation at the edge of chaos in recurrent neural networks. *Neural Comput*. **16**:1413–1436.

Chalupa, L.M., and J.S. Werner. 2004. The Visual Neurosciences. Cambridge, MA: MIT Press.

Debanne, D., D.E. Shulz, and Y. Fregnac. 1998. Activity dependent regulation of on- and off-responses in cat visual cortical receptive fields. *J. Physiol*. **508**:523–548.

Douglas, R.J., C. Koch, M. Mahowald, K.A. Martin, and H.H. Suarez. 1995. Recurrent excitation in neocortical circuits. *Science* **269**:981–985.

Freeman, W.J. 1975. Mass Action in the Nervous System. New York: Academic Press.

Gupta A., Y. Wang, and H. Markram. 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. *Science* **287**:273–278.

Häusler, S., and W. Maass. 2005. A statistical analysis of information processing properties of lamina-specific cortical microcircuit models, submitted. Available online as #162 from http://www.igi.tugraz.at/maass/publications.html

Jäger, H. 2002. Tutorial on training recurrent neural networks, covering BPPT, RTRL, EKF and the "echo state network" approach. GMD Report 159. http://www.ais.fraunhofer.de/de/publ/2002.html

Jäger, H., and H. Haas 2004. Harnessing nonlinearity: Predicting chaotic systems and saving energy in wireless communication. *Science* **304**:78–80.

Kaske, A., and W. Maass. 2005. A model for the interaction of oscillations and pattern generation with real-time computing in generic neural microcircuit models. *Neural Networks*, in press. Available online as #156 from http://www.igi.tugraz.at/maass/publications.html

Koch, C. 1999. Biophysics of Computation: Information Processing in Single Neurons. New York: Oxford Univ. Press.

Legenstein, R.A., and W. Maass. 2005a. Edge of chaos and prediction of computational power for neural microcircuit models. Submitted for publication. Available online as #166 from http://www.igi.tugraz.at/maass/publications.html

Legenstein, R.A., and W. Maass. 2005b. What makes a dynamical system computationally powerful? In: New Directions in Statistical Signal Processing: From

Systems to Brain, ed. S. Haykin, J.C. Principe, T.J. Sejnowski, and J.G. McWhirter. Cambridge MA: MIT Press, in press. Available online as #165 from http://www.igi.tugraz.at/maass/publications.html

Legenstein, R.A., C. Näger, and W. Maass. 2005. What can a neuron learn with spike-timing-dependent plasticity? *Neural Comp*. **17**:2337–2382. Available online as #154 from http://www.igi.tugraz.at/maass/publications.html

Maass, W. 1996. Lower bounds for the computational power of networks of spiking neurons. *Neural Comput*. **8**:1–40. Available online as #75 from http://www.igi.tugraz.at/maass/publications.html

Maass, W. 1997. Fast sigmoidal networks via spiking neurons. *Neural Comput.* **9**:279–304. Available online as #82 from http://www.igi.tugraz.at/maass/ publications.html

Maass, W. 2000. On the computational power of winner-take-all. *Neural Comput*. **12**:2519–2536. Available online as #113 from http://www.igi.tugraz.at/maass/ publications.html

Maass W., P. Joshi, and E.D. Sontag, 2005. Principles of real-time computing with feedback applied to cortical microcircuit models. In: Advances in Neural Information Processing Systems, Cambridge MA: MIT Press, in press. Available online as #164 from http://www.igi.tugraz.at/maass/publications.html

Maass, W., R. Legenstein, and N. Bertschinger. 2005. Methods for estimating the computational power and generalization capability of neural microcircuits. In: Advances in Neural Information Processing Systems, vol. 17, ed. L.K. Saul, Y. Weiss, and L. Bottou, pp. 865–872. Cambridge, MA: MIT Press. Available online as #160 from http://www.igi.tugraz.at/maass/ publications.html

Maass, W., T. Natschläger, and H. Markram. 2002. Real-time computing without stable states: A new framework for neural computation based on perturbations. *Neural Comput*. **14**:2531–2560. Available online as #130 from http://www.igi.tugraz.at/maass/publications.html

Maass, W., T. Natschläger, and, H. Markram. 2004. Computational models for generic cortical microcircuits. In: Computational Neuroscience: A Comprehensive Approach, ed. J. Feng, pp. 575–605. Boca Raton: Chapman and Hall/CRC. Available online as #149 from http://www.igi.tugraz.at/maass/publications.html

Maass, W., and E.D. Sontag 2000. Neural systems as nonlinear filters. *Neural Comput*. **12**:1743–1772. Available online as #107 from http://www.igi.tugraz.at/maass/ publications.html

Marmarelis, P.Z., and V.Z. Marmarelis. 1978. Analysis of Physiological Systems: The White-Noise Approach. New York: Plenum Press.

Pouget, A., and, T.J. Sejnowski. 1997. Spatial transformation in the parietal cortex using basis functions. *J. Cogn. Neurosci*. **9**:222–237.

Rieke, F., D. Warland, R. de Ruyter van Steveninck, and W. Bialek. 1997. Spikes: Exploring the Neural Code. Cambridge, MA: MIT Press.

Salinas, E., and T.J. Sejnowski. 2001. Gain modulation in the central nervous system: Where behavior, neurophysiology, and computation meet. *Neuroscientist* **7**:430–440.

Savage, J.E. 1998. Models of Computation: Exploring the Power of Computing. Reading, MA: Addison-Wesley.

Shepherd, G.M., ed. 1990. The Synaptic Organization of the Brain. New York: Oxford Univ. Press.

Shepherd, G.M., and C. Koch. 1998. Introduction to synaptic circuits. In: The Synaptic Organization of the Brain, 4th ed., ed. G.M. Shepherd, pp. 1–36. New York: Oxford Univ. Press.

Silberberg, G., A. Gupta, and H. Markram. 2002. Stereotypy in neocortical microcircuits. *TINS* **25**:227–230.

Sima, J., and P. Orponen. 2003. General-purpose computation with neural networks: A survey of complexity theoretic results. *Neural Comput.* **15**:2727–2778.

Swindale, N.V., D. Shoham, A. Grinvald, T. Bonhoeffer, and M. Hubener. 2000. Visual cortex maps are optimized for uniform coverage. *Nat. Neurosci*. **3**:822–826.

Thomson, A.M., and O.T. Morris. 2002. Selectivity in the inter-laminar connections made by neocortical neurons. *J. Neurocytol*. **31**:239–246.

Tsodyks, M. 2002. Neural circuits: Models of emergent functions. In: International Encyclopedia of the Social and Behavioral Sciences, Elsevier Science, http://www.iesbs.com

Left to right: David McCormick, Hannah Monyer, Javier DeFelipe, Rafa Yuste, Jean-Pierre Changeux, Eörs Szathmáry, Christoph Michel, Maria Blatow, Wolfgang Maass, and Yves Frégnac

19

Group Report: Neocortical Microcircuits

UPs and DOWNs in Cortical Computation

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INTRODUCTION

A remarkable feature of the vertebrate brain is the key role played by cerebral cortex in the versatility of computation during cognitive operations and the adaptive control of the organism's actions when immersed in a novel environment. In particular, as advocated by William James as early as 1890, there seems to be an almost quantitative "fit" between the preeminence of cerebral cortex and the "complexity" of the cognitive repertoire specific to each species. Since then, searching for regional cortical uniqueness versus uniformity has been considered a primary axis of study. Two concepts of cortical organization are classically opposed: on one hand, phrenology and now functional brain imaging have given some credence to the delineation of specialized "organs of the mind" by singularizing regional variations of anatomy, metabolism oxygen consumption, and hemodynamic flow across the cortical mantle (Spurzheim 1824; Dehaene, Dehaene-Lambertz, and Cohen 1998). It has been argued that this parcellation in distinct functional areas, and the specialization of the columnar motifs that pave the cortical gray matter sheet, may be entirely genetically determined (Rakic 1988). On the other hand, network statistics reveal a number of structural regularities, which, to a certain extent, are found repeated across the whole cortex (Bok 1936; Sholl 1956; White 1989). Since then, numerous studies have tried to look for canonical building blocks and to define quantitative criteria for an area-specific articulation of these elementary processing units. The ultimate goal is to decide which ones, between the most probable intra- and inter-area

interactions derived from cortical anatomy and physiology, form a semantic basis for implementing specialized cortical computations.

A supplementary twist in this debate is the possibility that a diversity of building blocks coexists in the same anatomical network. Dynamic selection processes may enforce order on synaptic interactions, ensuring formation and dissolution in time of specific functional circuits (Woolsey in White 1989). It is therefore likely that the reductionist dream of molecular biologists who propose that "from the DNA sequences stored *in silico*, one may be able to compute the main features of the species-specific functional organization of the brain" is far from being fulfilled. Not only additional quantitative markers at the molecular and genomic levels are needed, but reproducible state-dependent contexts must be defined before one may seriously envision dissecting out waxing and waning forests of nested circuits during the time-course of a mental event.

This chapter summarizes our group's discourse on neocortical circuits at the 93rd Dahlem Workshop. We begin with a conceptually driven discussion and address the problem of decomposition of cortical structure, function, and computation in canonical elements. The comparison of taxonomies established at various integration levels raises the question of continuum versus clusterization; new techniques are now available to search in real-time for the transient switch-on of cortical microcircuits not only within, but also across anatomical microcolumns. Thereafter we focus on the identification of the format of the information processed by the cortex and the relevance of a multiplicity of temporal scales in coding schemas. Possible links are presented between the horizontal spread of depolarized "UP" states in cortical neurons, the transient synchronization of active assemblies repeated according to stereotyped temporal motifs, and the recent reinterpretation of the existence of temporal sequences of fixed microstates in the dynamics of global EEG maps (initially observed by Lehmann 1971). Since high conductance states appear to be generated *in vivo* by intense synaptic recurrence in the cortical network, models and electrophysiological experiments based on dynamic clamp techniques are now testing the possibility that attentional processes control cortical gain by changing membrane properties such as membrane potential and its synaptically driven variance. A third issue is more hypothetical and refers to plausible neural architecture for the emergence of cognition, and even access to consciousness. The last section illustrates one feature that is specific to the think-tank sessions as practiced at a Dahlem Workshop: interdisciplinarity enriches the field of study by borrowing concepts, tools, and analogies from other disciplines. New paradigms for the study of brain computation can be suggested on the basis of experience provided by computer science and theoretical physics. Evolutionary considerations using language as an example can also validate certain circuit architectures maintained through phylogeny. Even though the reader will not find decisive conclusions on the elusive existence of cortical microcircuits in this chapter, we hope to provide a fair view of consensus, controversies, and challenges that may guide future research.

CONCEPTS: CANONICAL DECOMPOSITION OF BRAIN STRUCTURE, FUNCTION, AND COMPUTATION

Although its existence is not guaranteed, modularity in brain organization can be studied at different structural levels, ranging from molecular ensembles to sets of interconnected neuronal networks. Its functional expression can also be searched for on different timescales in the nonrandomness of temporal patterns of activity arising from gene expression to coordination of neural activity between cell assemblies.

A first issue is to find ways of *reducing the level of complexity* of the biological system under study (membrane compartment, cell, network, map) by detecting stereotypes in the space and time domains. A second issue will be to show that the whole system organization and functional repertoire can be integrated in a hierarchy of elementary building blocks. If one accepts the possibility that such decomposition exists and, in addition, is linear, the functional output can be predicted from the convolution of the transfer function of each basic module and its input. A cartoon view of these concepts of modularity and segmentation, detailed in Figure 19.1, would be to imagine that the cortex, whatever its functional specificity, is composed of an array of identical modules replicated all over its surface (see Figure 19.1, case "1 $\times$ N"). The specialization of cortex into identifiable sensory, association, and motor areas would result from specific combinations of basis modules. Another possibility is that the building block set is heterogeneous (see Figure 19.1, case "N $\times$ 1"). If, in addition, some cross-talk occurs between elementary processes (see Figure 19.1, cases "Overlaid" and "Nested"), a nonlinear interaction is expected between modules and has to be taken into account. This can be done by defining additional binding principles and identifying specific relational architectures.

Another issue is the *preservation of the elementary function* of each module while progressively segmenting the whole network. The "invariance-by-segmentation" property may not be verified in highly recurrent networks, such as cortex, where an elementary processing module may be nested in a hierarchy of subnetworks of variable recurrence. Cutting one of the loops might alter, or even cancel, the global functional attributes of the cortical network. The case of the primary visual cortex illustrates the principle of recurrence. Most connections originate from within cortex even for cells that receive the direct impact of extrinsic inputs, a feature which has long been underestimated. For instance, a layer IV spiny stellate cell in cat area 17 receives only 6% of feedforward thalamo-cortical inputs, whereas 94% correspond to recurrent and feedback connections (Ahmed et al. 1994; Binzegger et al. 2004). Further studies may show that it is necessary to envision primary visual cortical modules not only as highly recurrent networks but also as encapsulated in a larger ensemble of thalamo-cortico-thalamic and long-distance corticocortical loops. Continuing to study the properties of cortical cells independently of the anatomical context

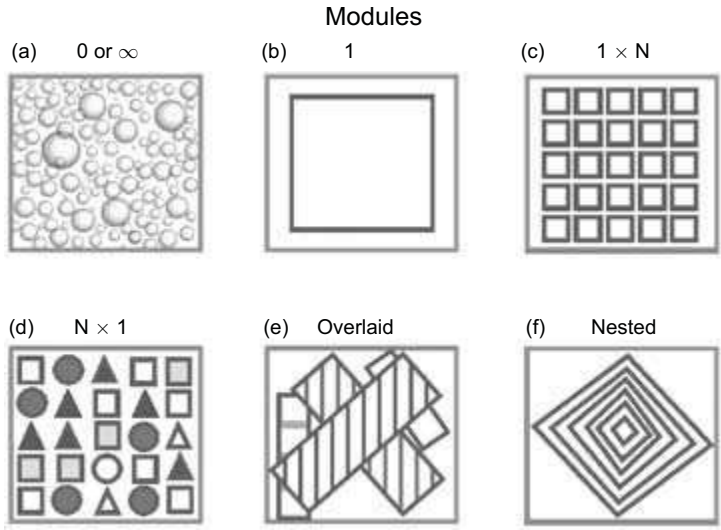

Figure 19.1 Modules: Several theoretical possibilities may be explored in the decomposition of cortex into an array of basic elements at the structural and/or functional levels. (a) "0 or ∞": These elements may be infinite in number, which is the equivalent of saying that, at the lowest level of organization, each and every element is unique and that no module is replicated. (b) "1": The cortex is the module itself. (c) "1 × N": Only one type of elementary module exists, replicated *n* times spatially all over cortex. This uniform tiling would not exclude the variant that a local combination of several basis modules would be responsible for the specialization of cortical areas into identifiable sensory, association, and motor areas. (d) "N × 1": The building block set is heterogeneous but finite. (e) "Overlaid": The building blocks are not independent and share elements. (f) "Nested": The modules are defined at different levels of integration and can be integrated in a compositional way in nested architectures.

of the recurrent network in which they are embedded may falsify our search for understanding the genesis of the specificity of cortical computations.

Canonical Structural Circuits (Static Circuits)

The search for microcircuits requires looking at repetitions of elementary units in terms of constituents (molecular–anatomical level) and invariant topological motifs in the connectivity pattern between these constituents. The most intuitive version of canonical circuits can be found in the radial columnar architecture of the cortex and its structure in six layers. In the case where the input projects in a regular topographic way onto the laminar plane and the location of cortical receptive fields is invariant along the cortical depth, the temptation is to collapse the 3D network into a 2D network, where each integration point represents an elementary cortical microcolumn. One of the most studied examples is the

cortical mosaic of input–output modules juxtaposed in the somatosensory cortex of some rodents. Each module embodies the imprint of the parcellation of the sensory periphery onto the cortical field; each cortical barrel represents one individual whisker in the somatosensory cortical matrix organized in rows and arcs and has the same respective location as the whisker in the snout (Woolsey and Van der Loos 1970). Other mapping examples, more continuous in nature, can be found in the visual cortex, although some debate was initially raised concerning the mosaic/distributed aspect of the global cortical network (Albus 1975).

Canonical Activity Processes (Dynamic States)

The Metacolumn Concept

In the Hubel and Wiesel model of the "hypercolumn" in primary visual cortex (Hubel and Wiesel 1963), the anatomical extent of the circuit should roughly correspond to the cortex volume activated by an impulse-like input (cortical spread function). However, the use of voltage-sensitive dye imaging techniques or intracellular recordings *in vivo* shows that the visual activity evoked by a point or bar-like stimulus spreads far beyond the classical radius of the anatomical column defined by vertical integration process along the cortical depth and extends laterally over long distances (Grinvald et al. 1994; Frégnac and Bringuier 1996; Bringuier et al. 1999). On distinct grounds, theoreticians have introduced the functional concept of "metacolumn" (see Figure 19.3, bottom left), where, in addition to the radial column characterizing vertical integration, a chunk of cortex spreading horizontally in superficial layers has been added in order to provide the contextual intracortical input required for completing the functional integration (Somers et al. 1998). Without this extended environment, the functional selectivity of the cortical module (e.g., its direction orientation selectivity or preference) would be lost.

The Temporal Signature of Spike Trains

Independently of the spatial location of the elements in the cortical tissue, another approach is to develop in time the activity of each member of the recruited assembly and search for some form of invariance in the temporal patterns of spike trains. Such stereotypy can be expressed either in a stimulus-locked fashion (time coding) or in the phase-relationship of spike activity across the different cells forming the assembly (relational coding). Multiple simultaneous recordings in the awake behaving monkey motor cortex have shown replication above chance level of temporal motifs composed by composite intervals between spikes of different units with a precision in the 2 ms range. The occurrence of such motifs has been found to be correlated with the nature of the

behavioral task (Go vs. No-Go in Vaadia et al. 1995) and could carry information related to the probability of expected reinforcement (Riehle et al. 1997). Stereotypy in time could then represent the signature of canonical operations specific to the relational (binding) topology of the activated graph, but not of the anatomical identity and location of the elements composing its nodes. This point will be developed further below (see section on FORMAT OF INFORMATION AND RELEVANCE TO TEMPORAL SCALE).

Canonical Computations and Compositionality Issues

Whatever the chosen level of integration, the next question is: How many modules can be extracted? Possible answers, detailed in Figure 19.1, are none, one (the cortex), many (the columns), or an infinity (the cell). The so-called "hypercolumn" can be seen as an example, in primary visual cortex, of a unitary module composed by the grouping of a finite set of anatomical columns whose boundaries are defined by the spatial spread of thalamic afferents representing the same point in space seen through each eye (Hubel and Wiesel 1963). However, the task becomes more daunting if the aim is to extract the subcircuits responsible for each elementary operation performed by the cortex. A group of neurons collaborating to perform one operation may not necessarily collaborate to perform a different operation (Swindale et al. 2001).

In the case where the answer is not "one" but "many," is it possible to look for compositionality, that is, an alphabet and a grammar (Cavanagh 2003)? Can we predict the network performance on the basis of a functional syntax combining chains of elementary processes? This problem is compounded by the fact that multiple operations can be multiplexed in time in the same network. For instance, at a given point in time the spiking of a given cell may participate in two parallel computations, which engage transiently the same cell in different assemblies. An interesting theoretical development would be to search for scale-free architectures where similar binding rules operate at different integration levels (nested architectures). What applies between cell members of the same assembly may apply to interaction rules between compartments of a given dendrite (Mel 2003).

Validation of a Canonical Process/Circuit/Computation

Validation should be performed by comparing circuit stereotypes across different cortical areas within one species as well as across homologous cortical areas within different species. Phylogenetic and ontogenetic perspectives can serve to strengthen the argument of equivalence across circuit types and help define the functional attributes of new circuits that emerge during development and evolution (see section on EVOLUTIONARY CONSIDERATIONS USING LANGUAGE AS AN EXAMPLE).

TAXONOMY WITHIN AND ACROSS INTEGRATION LEVELS: CONTINUUM OR CLUSTERS

Continuum of Variability or Categorization of Diversity?

One may, or may not, believe in canonical modules. In spite of the generally limited confidence in such matters, the measure of variability against generic diversity should be evaluated with quantitative methods, not only across constituents within a given cortical area, but also across different functional areas and across species. In the latter case, one must accept some stratification hypothesis with evolution, where abrupt changes of primary principles are rarely observed, and refinement, elimination, or addition tend to be the rule. This argument, for instance, may advocate the use of the tree shrew model, where numerous functional streams afferent to V1 remain more segregated in the cortex at the anatomical level than in other mammalian species, and where the detection of a correlation with function and laminar location may be facilitated (Fitzpatrick 1996; Mooser et al. 2004).

Over the past few years, more attention has been given to inhibitory processes in the shaping of cortical functional selectivity, and a significant set of data reveals the computational diversity that may exist in inhibitory circuits (*in vitro*: Gupta et al. 2000; Monyer and Markram 2004; *in vivo*: Monier et al. 2003). Concerning the taxonomy of GABAergic interneurons, the following questions are still a matter of debate:

1. Is there a finite number of interneuron subtypes, and what criteria are necessary to classify GABAergic interneurons?
2. What is the minimal set of criteria that can help identify distinct subpopulations of GABAergic interneurons?
3. Why would so many types of interneurons be useful?
4. What approach allows for a systematic study of identified interneurons?

During the Dahlem Workshop, our group partially answered the first and last questions; however, no decisive consensus was reached for the others.

Morphological Taxonomy

There is great diversity of interneurons based solely on the morphology of their somata and of their dendritic and axonal arborizations. There is, however, no established convention for assessing which morphological characteristics of a neuron are essential to pertain to a given cell type or, in other words, which morphological differences are functionally important. Nevertheless, a positive consensus was reached for the first question, since certain interneurons can be recognized by their unique morphological characteristics, or on the basis of their patterns of axonal arborization, or the synaptic connections they establish with other interneurons and/or with pyramidal cells. Further, interneurons are not

solely connected by point-to-point chemical synapses and are often electrically coupled within specialized gap junction networks (Galarreta and Hestrin 2001).

Even if interneurons are differentiated into subtypes, some either lack or display a great variability profile when compared across different species. One of the best studied examples is the *double bouquet cell*. There is considerable interest in studying this interneuron, because it forms a widespread and regular microcolumnar structure spanning from superficial to deep layers, and because it appears to represent a key component of the minicolumnar organization of the primate neocortex (see section on SEARCH FOR CORTICAL MICROCIRCUITS). However, these neurons are less numerous in the cortex of other species or may even be absent (e.g., in mouse and rat, although this is still a matter of debate). In addition, there are significant variations in the number of neurons and the proportion of excitatory (glutamate) and inhibitory (GABA) neurons and synapses within the minicolumn in different areas and species (DeFelipe et al. 2002).

Molecular Determinants and Multiparametric Taxonomy

Recent studies have extended the dimension to the classification search. Experts in the field still think that GABAergic interneurons can be further subdivided into distinct subtypes if sufficient criteria are considered and cross-correlated. The most frequent parameters that have been used so far are morphological features (e.g., soma shape, dendritic and axonal arborization, axonal targets), neurochemical markers (calcium-binding proteins, neuropeptides, neurotransmitters or their synthesis enzymes), intrinsic electrical properties (e.g., firing pattern, firing frequency, action potential width and amplitude, input resistance), synaptic dynamics (connectivity and its plasticity, decay time constants of EPSPs and IPSPs), as well as a specific repertoire of expressed proteins (e.g., ion channels, receptors).

The minimal number of required criteria has not yet been assessed but most experts agree that it will vary depending on which cell types are studied. Thus, fast-spiking cells can be predicted to be somatostatin-negative, since this marker has not been found in this frequently studied cell population so far. However, they can also be subdivided in subclasses if other parameters are taken into account, for example, axonal arborization and synaptic properties. Recent attempts at correlating firing properties with protein expression corroborate the existence of clear-cut GABAergic interneuron subtypes (Toledo-Rodriguez et al. 2004; Monyer and Markram 2004). Present data also suggest the existence of molecular determinants underlying oscillatory and synchronous network activity and lead to the conclusion that different types of interneurons may subserve distinct functions, for example, by participating in the generation of oscillatory activity in different frequency bands (Blatow et al. 2003; Whittington and Traub 2003). This may have a decisive impact on controlling the precision of spike timing (see below) and more studies on this issue are expected *in vivo*.

METHODOLOGICAL CONSTRAINTS IN CLUSTER ANALYSIS

The use of multiple criteria raises an interesting combinatorial issue. As illustrated in Figure 19.2, the assumption is made that all diversity of the repertoire should be found in a "hypercolumn cube" of $1 \times 1 \times 2$ mm, which is a rough estimate of the volume of cortex required to process one point in visual space through both eyes and a complete preference set of orientation filters. The number of classes given in Figure 19.2 is based on the most recent studies performed *in vitro* (Toledo-Rodriguez et al. 2004). If the profiles observed for each classification type (anatomical, genomic, electrophysiological) were to be independent, the basic element of the neocortical microcircuit can be considered the cell itself and its singularity, since the number of neurons and potential categories are roughly comparable! Also, armies of postdoctoral fellows, working *in vitro,* might get depressed by the simple thought experiment of guessing the number of cells to be recorded before reaching statistical significance level.

Cluster analysis provides a quantitative method with which to measure in a multidimensional space how similar neurons are to one another. The group of Rafael Yuste has recently applied this approach to a population of neocortical interneurons from mouse primary visual cortex with the goal of examining how many distinct classes of interneurons exist (Dumitriu et al., submitted; Yuste 2005). The sample of interneurons included parvalbumin (PV)-positive, somatostatin (SS)-positive, and neuropeptide Y (NPY)-positive cells, as selected from transgenic animals expressing GFP under the control of these three promoters. These neurons were patched and their intrinsic electrophysiological parameters measured, as well as the time constants of the spontaneously received EPSPs and IPSPs. The neurons were also filled with biocytin and reconstructed morphologically after fixation. For each neuron, a series of ~100 different morphological parameters were measured. The morphological and physiological

Criteria	# Classes	
Anatomy	Types	3
	Subtypes	150
Electrophysiology		20
Multiplex RT-PCR		20

$3 \times 150 \times 20 \times 20 = 180{,}000$

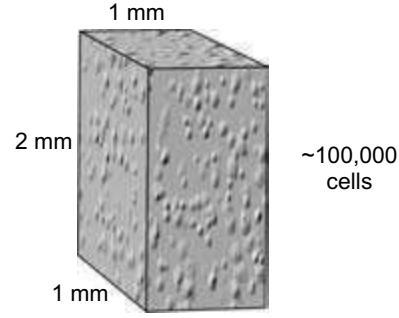

Figure 19.2 Taxonomy and the hypercolumn: The volume of the cortical tissue chunk is the size of a functional hypercolumn (Hubel and Wiesel 1963). The number of neurons is roughly of the order of the number of classes (180,000) based on anatomical, electrophysiological, and genomic criteria (see text for the choice of parameters).

parameters were then used to generate two cluster trees: one based on the morphology and the other on the physiology. Interestingly, both trees had three major branches, which corresponded quite accurately to the three groups of PV, SS, and NPY interneurons.

From these results, it can be concluded that at least *three* distinct different classes of neocortical interneurons exist in mouse primary visual cortex. Further, there is a correspondence between the biochemical, morphological, and electrophysiological characteristics of the neurons within those groups, since the clusters found with the electrophysiological analysis can be used to predict the morphological clusters and they correspond to the expression of these three marker proteins.

One disadvantage of cluster analysis is that it always results in clusters and does not provide a natural cutoff in the classification, which in principle can be pursued with subsequent subdivisions until each cluster has a single individual. At the same time, the use of independent measurements with which to cluster a data set can help in distinguishing important clusters from the noise. Overall, the arguments in favor of the real existence of distinct classes of neocortical neurons are very compelling in the case of neocortical interneurons. There are striking correlations between the morphologies of the axon and dendrites, the firing patterns and spike and AHP characteristics, the EPSP and IPSPs kinetics, the synaptic dynamics, the coupling through gap junctions to neurons of the same class, and the expression of distinct protein markers. It is very probable, like the interneurons in the spinal cord, as demonstrated by Jessell and colleagues (Tsuchida et al. 1994), that different classes of neocortical interneurons could differentiate under the control of different promoters and play specific circuit roles. In general, if the circuit is built with specific elements, it appears absolutely essential to come to terms with this diversity in order to understand the function of the circuit.

Standing Issues

To a certain extent, numerous studies, whether at the anatomical or functional level, have for a long time erased the variability content of their data by looking for average morphological structures or average temporal response profiles, without analyzing the computational impact of possible diversity. This makes the comparison between previously treated databases obtained in distinct laboratories difficult. No effort has been made to reconcile the classification methods and criteria used by different groups. Therefore, it is important that some benchmark is proposed to validate the classification of data. An international database is clearly needed. The criteria of classification should be widely accepted and regularly updated (see the collective document which has been proposed by 35 scientists at http://www.columbia/cu/biology/faculty/yuste/petilla), taking into account the rapidity of the techniques in that field.

The multiplex RT-PCR technique has limitations. The first is quantitative and concerns the existence of high probabilities of false negatives. Second, mRNA measurement in the slice looks like a "photograph" made after a massive disturbance of activity imposed by the slicing process itself, which may result in spurious activity-dependent regulation of gene expression that may vary according to the experimental protocol. A third, fundamental issue is that one should not limit oneself to cytoplasmic mRNA harvesting. The search should be extended to the proteome and membrane-bound proteins in order to establish the cell-by-cell distribution of receptor and ions. In other words, it is likely that multiplex RT-PCR will not give access to the "molecular shape" of the neuron. A question of importance in terms of cellular computation remains the subcellular distribution of the balance between GABA and glutamate receptors across the dendritic compartments.

Genetic approaches that allow the marking of interneuron subclasses with an *in vivo* fluorescent protein are a promising avenue that is being taken in order to identify even rare subtypes in the slice preparation and *in vivo*. They will certainly promote the systematic study of GABAergic interneurons at the cellular and system levels.

SEARCH FOR CORTICAL MICROCIRCUITS: WITHIN OR ACROSS COLUMNS?

Within Columns (Vertical)

The issue of whether or not there is a canonical microcircuit in the neocortex is of the utmost importance. The possibility of a common transfer function performed on any cortical input could be the equivalent of a DNA helix model of the brain. As conceptualized earlier, the canonical microcircuit hypothesis can be articulated as the existence of a common single operator (or transfer function) of cortical function, one which would be similar in different cortical regions and in different species. This hypothesis was first explicitly articulated by Hubel (Hubel and Wiesel 1974) and has been most developed in the writings of Douglas and Martin (1991, 2004; Douglas et al. 1989).

Brief History of the Columnar Consensus

In spite of the diversity of neuronal elements and the profusion of connections within the cortical network, one should recognize that there are strong arguments in favor of a canonical microcircuit, especially linked with phylogeny and ontogeny. Like in other systems in the evolution of the body plan, it is likely that the neocortex arose by manifold duplication of a similar circuit module. The relatively short evolutionary history of the neocortex, together with the prodigious increase in size it has experienced in mammals, make this idea appealing. Also,

developmentally, all cortices of all animals arise through a very stereotypical sequence of events: from neurogenesis in the ventricular zone, through migration along radial glia, depositing of neuroblasts in cortical layers, and emergence of axons, dendrites, and dendritic spines. These events occur in some cases with nearly identical timing in different parts of the cortex and in different animals, so it is not unreasonable to argue that they result in the assembly of an essentially identical circuit.

The Radial Microcolumn

Anatomically, the presence of vertical chains of neurons defining small columnar structures has been noted at least since Lorente de Nó (1938). Cyto- and myelo-architectonic studies from even earlier dates show presence of vertical aggregates of neurons and vertical bundles of axons. This radial arrangement is frequently referred to as the micro- or minicolumnar organization. Similar bundles of apical dendrites have been noticed more recently using a variety of techniques. Therefore, this arrangement defines an anatomical module, consisting of a vertically oriented group of interconnected cells, which are contained in a vertical cylinder of tissue with a diameter ranging approximately from 25 to 50 μm (depending on the cortical area and/or species). These structural modules appear in many different parts of the cortex in many different species (see schematized representation in Figure 19.3b). Dendritic bundles of apical dendrites of pyramidal cells have been described in various areas of the mouse, rat, rabbit, cat, and monkey, the size and number of dendrites forming the bundles depending on the cortical area and species being variable (e.g., Peters and Walsh 1972; Fleischhauer 1978). Since there are significant variations in the number of neurons as well as in proportion to the excitatory (glutamate) and inhibitory (GABA) neurons and synapses within the minicolumn in different areas and species (DeFelipe et al. 2002), we can conclude that the radial minicolumns should be considered dominantly as regularly distributed vertical aggregates of pyramidal excitatory neurons.

The Functional Column

The basic functional element of the neocortical microcircuit can be defined primarily by the vertically dominant integration flow of activity evoked by thalamic input (see Figure 19.3c). The electrophysiological recordings since Mountcastle and Edelman (1982) and Hubel and Wiesel (1977) have emphasized the invariance of receptive fields along vertical electrode penetrations, in terms of spatial location in the visual field and orientation preference property. These pioneering studies led to the specific proposal of functional columns of different scales. The macrocolumn is defined as a complex processing and distributing unit that links a number of inputs to a number of outputs via overlapping internal processing chains (minicolumns) (Mountcastle 1997). One should

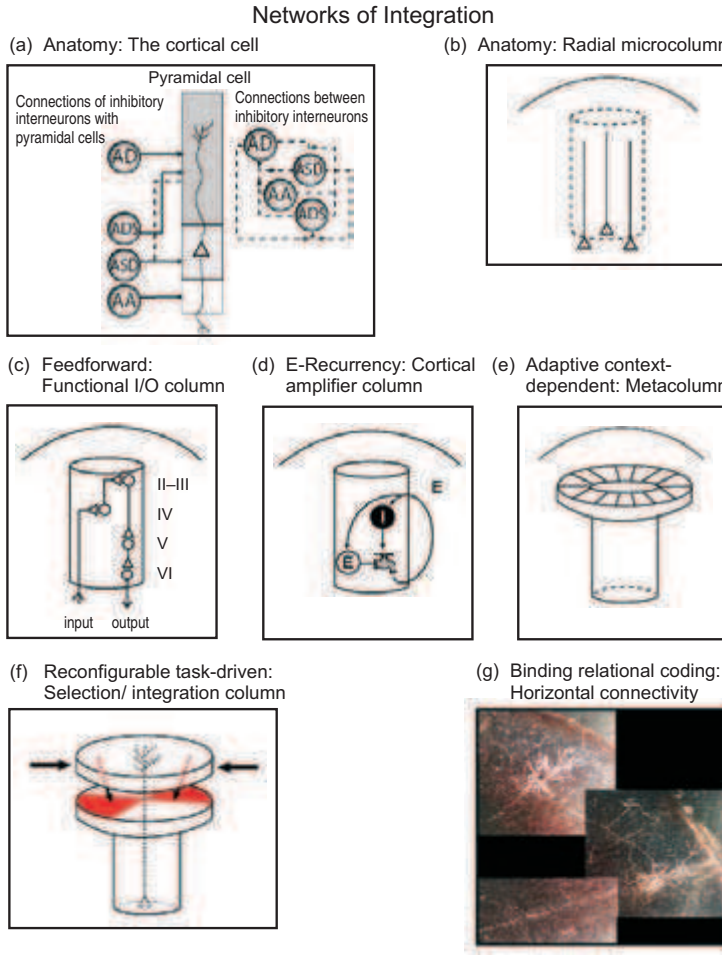

Figure 19.3 Networks of integration. (a) The cortical pyramidal cell and its membrane compartments represent an elementary site of synaptic convergence. (b) Bundles of axons of pyramidal cells form radial microcolumns. (c) One of the best-studied input–output circuit characterizes the serial processing of layer IV afferents by first-order targets, the stellate cells in layer IV. After a series of successive relays in layer II/III and layer V, these terminate on layer VI neurons, which send their axons out of the functional column (Gilbert and Wiesel 1979). (d) The canonical microcircuit exemplifies the high level of recurrence of excitatory local connections whereas the inhibitory interneurons control the gating of the avalanche of excitatory amplification (Douglas and Martin 1991). (e) The concept of metacolumn, introduced by Somers et al. (1998), corresponds to the network influence carried via long-distance horizontal connections in the supragranular layers (see g), that needs to be added to the column to predict its context-dependent behavior. (f) The hypothesis of selection of computational circuits (red volume) by the neuromodulatory action of ACh fibers running in layer I. (g) Inverted contrast picture of two biocytin-labeled layer II/III pyramidal cells connected by horizontal axons (Frégnac and Friedlander, unpublished).

note, however, that the definition of the functional column applies to the input–output circuit formed exclusively by serial excitatory links from layer IV (input layer) to layer VI (one of the output layers). The laminar relay description (IV → II–III → V → VI) within the column is based on the assumption that axons are connected to neurons whose somata were located in the layer to which the axon projects (Gilbert and Wiesel 1983).

Standing Controversies

Although most Cajal lovers dream of wandering in a forest of pyramidal cells, recognizing here and there a repetitive anatomical motif, there are perhaps even more compelling reasons "against" than "for" the "columnar" canonical hypothesis. It is indeed hard to imagine that there is a common denominator in all of the different computational problems that the cortex is solving. In some cases, these problems are essentially mathematically irreducible, such as the 3D visual processing, as compared with auditory speech perception, for example. Also, the exact nature of the structure of the cortical modules is elusive to define. Anatomical techniques do not reveal any clear borders between modules, and physiological approaches reveal a combination of maps superimposed onto one another with different metrics, such as orientation ocular dominance or spatial frequency (Basole et al. 2003). Recent intracellular *in vivo* recordings in V1 cortex show that the computation of orientation preference, thought to be internalized in columnar modules according to the feedforward model of Hubel and Wiesel, is in fact the result of a diversity of combinations of excitatory and inhibitory inputs. This diversity reflects mostly the anatomical nonuniformity of the intracortical input context provided by the orientation map ("metacolumn" concept considered above; Figure 19.3e, f) in which the cell is embedded (Schummers et al. 2002; Monier et al. 2003; Frégnac et al. 2003). Finally, the detailed anatomical comparison of cortical neurons sampled from different regions reveals that each cortical region is endowed with specific subtypes of pyramidal neurons, as revealed by Elston, DeFelipe, and Yuste (Elston et al. 2001; Elston and DeFelipe 2002; DeFelipe et al. and McCormick and Yuste, both this volume).

Across Columns (Horizontal)

As noted earlier, a strong historical bias can be found in favor of the description of modular circuits respecting the laminar organization and organized along the depth (vertical) dimension of the gray matter. Similarly, at the functional level, a strong bias can be noted in the elucidation of feedforward sequential streams in processing. An analogy can, however, be made with computers and the problem of minimization of the wire length (Peters and Kaiserman-Abramof 1970; Chklovskii et al. 2002; Mitchison 1991; Mead 1989). To avoid a complete

connectivity pattern and reduce the physical size of the global system, a hardware configuration often used is to stack-over interface bus-cards, each dedicated to input only or output only. In that respect, the vertical dimension does not carry processing, and only the lateral dimension is used to wire the computing architecture. This analogy suggests that, rather than looking for vertically organized columns, one should concentrate on the pattern of horizontal connectivity (Figure 19.3e, f) to characterize the functional specialization of the cortical network under consideration.

Excitatory horizontal connectivity from visual to prefrontal cortex exhibits a patchy layout: the interpatch spacing is roughly double that of the patch diameter. This patchy architecture has been studied extensively in primary visual cortex and shows a strong correlation between anatomy and function. Cells that are connected through long excitatory links tend to belong to columns with the same functional preference. Some authors, however, have moderated the impact of the principle "those alike tend to wire together." This schema neglects the spatial organization of axons and dendritic structure of the target cell. The matching of the composite size of the axonal terminal distribution from the presynaptic cells with that of the dendritic spread of the target cell could be the result of an optimization process maximizing the diversity of inputs collected by a given neuron (Malach 1992).

Several arguments can be listed to support the fit between the function and the anatomy of the horizontal network. During development in strabismic cats, anomalous horizontal connectivity links are formed between distant cortical territories corresponding to the same eye-dominance (Schmidt et al. 1997; Trachtenberg and Stryker 2001). Another example can be observed with a sensory substitution protocol imposing a rewiring of the input to the auditory thalamus at an early stage of development. If visual input is provided to auditory thalamus at that time, the auditory cortex develops a visual competence and an orientation preference map. The horizontal connectivity anatomy in the rewired A1 cortex resembles that of a control area V1, in terms of anteroposterior/mediolateral biases, and not to that of a normal auditory cortex (Sharma et al. 2000). To progress in this direction, work must be undertaken to characterize the factors, linked with activity and the sensory code, that determine the number of patches, the extent of their distribution, their input distribution, and their output distribution, both during normal and abnormal development.

Standing Consensus

The cortex looks like a multifaceted structure where some stratification and crystal-like regularity is apparent, depending on the view angle (vertical, horizontal) and the nature of the module for which one is looking (anatomical, functional). To the dismay of most experimenters, this remarkable versatility of

changing its crystalline motif adapts to the computational task on demand. In other words, cortical modules appear highly reconfigurable, and the autonomous structural entity that forms the grain of the lattice has a virtual boundary defined by the nature of the computation. Figure 19.3f illustrates a recent hypothesis made in favor of a reconfigurable selection network, where "the superficial layer neurons within and among patches, and within and among areas, cooperate to explore all interpretations of input and to select an interpretation consistent with their various subcortical inputs" (Douglas and Martin 2004). More work is also needed to elucidate the controversial role attributed to various ascending neuromodulatory influences and the still mysterious or controversial implication of layer I.

FORMAT OF INFORMATION AND RELEVANCE TO TEMPORAL SCALE

To extract computational steps in network processing, most experimenters and theoreticians have focused their attention on measures derived from spike activity, since this is the component of the neuronal integrative process that is broadcast through axons and synapses to the rest of the network. The most commonly used measures are based on:

- The *rate of action potential* generation per relevant unit of time. The relevant unit of time in terms of sensory coding is thought to vary from a few tens of milliseconds for processing simple features to several hundreds in the case of the construct of a mental percept.
- The *precise timing of action potentials* during this unit of time. Temporal reproducibility of stimulus-locked activity in response to a continuous flow of full field patterns gives some indication of the precision in spike occurrence time coding. In such stimulation context, precision, at least in retina and thalamus, narrows to the 2–5 ms range (Reinagel and Reid 2000; Frégnac et al. 2005). Associative forms of synaptic plasticity in cortical networks have also been shown to depend on the temporal order and delay between pre- and postsynaptic activities, with a precision in the order of a few ms (Markram et al. 1997).
- The spatiotemporal distribution of the activity in the network and the relative phase of firing between cells of the same assembly (Abeles and Gerstein 1988).

Although the following arguments concern coding by spike activity, it is important to note that recent efforts have been carried out to retrieve network state dynamics and information transfer measurements from the analysis of membrane potential trajectories of cortical cells (intracellular recordings; see reviews in Shapley et al. 2003 and Frégnac et al. 2003) and from optical imaging of

supragranular layer activity (voltage sensitive dyes: see, e.g., Arieli et al. 1996; Sharon and Grinvald 2002).

Rate versus Time Coding

Experimental support for a neuronal doctrine based on rate coding (Barlow 1972) is found in the stability of cortical measures of feature selectivity when using, as the output signal, the rate of discharge or total number of spikes evoked as a function of the orientation of the stimulus in V1 (Hubel and Wiesel 1962) or the direction of the planned movement in M1 (Georgopoulos et al. 1986). Despite this evidence, for the past ten years there has been a growing claim that information is also encoded in the timing structure of spike trains of single neurons. The study of statistical moments of higher order than the mean shows evidence for temporal precision in the order of a few milliseconds (review in Abeles 1991). Thus, multiple coding schemes may coexist in spike train patterns, accounting for different aspects of the functional dynamics of cortical networks. The prevalence of time versus rate coding in the same structure could depend on several factors, such as the density and statistics of the input regime and its associated computational load, the context of sensory adaptation, the internal state of the cortex (e.g., level of desynchronization in the EEG), and the dynamic regime imposed by the balance between recurrent excitation and inhibition.

Despite four decades of research characterizing the response properties of sensory neurons in primary cortical areas, we still do not have a good picture of how cortical neurons really operate under realistic conditions (i.e., how, for instance, natural scenes are encoded by cortical activity patterns). Much of our current knowledge is derived from experiments using reduced stimuli (i.e., impulse-like stimuli, such as spots, white noise, or sinewave gratings for the visual system). The main problem with this is that under the continuous influence of the feedforward drive and massive recurrent intracortical activity produced by natural images, cortical neurons are forced into a high conductance dynamic regime where their behavior may become highly nonlinear. More work is needed to compare the actual subthreshold and spiking activity of sensory cortical neurons in response to natural scenery movies or continuous sound tracks to predictions based on linear estimates of receptive field properties established by conventional methods.

The Synfire Chain Signature of Cortical Songs

Independent of the spatial location of the elements in the cortical tissue, another approach to describe a functional assembly is to develop the activity of each member of the recruited assembly in time and look for specific temporal cross-relationships. After more than twenty years of continuous research, Abeles and his group identified the replication above chance level of temporal motifs of a

few hundred milliseconds of total duration, composed by a chain of feedforward and recurrent excitations (Abeles 1982, 1991; Vaadia et al. 1995; Rieke 1999). These temporal motifs can eventually be found in high-order statistics of the spike train of a single cell, since the same element of the synfire assembly can participate several times in the activation chain. A continuous version of these discrete patterns has been reported recently in intracellular whole-cell recordings in voltage clamp mode *in vitro* and in intracellular current clamp recordings *in vivo* (Ikegaya et al. 2004), although the functional significance of such events remains to be clarified. Several features are remarkable, in the sense that these motifs become more precise in the timing of individual action potentials with the repeats and can sometimes bind transiently to each other. This last finding is reminiscent of the theoretical prediction that suggests compositionality of synfire chains through activity-dependent plasticity, which would affect the finally stabilized probability of connections between cortical cells (Delage 1919; von der Malsburg 1981; Bienenstock and Doursat 1991). Support for this view could eventually come from the morphological study of branching patterns of dendrites and axons, considered here as a read-out of the past association processes (Bienenstock 1996). An extreme view would be to consider that the temporal motifs of cortical spike trains form a "cortical song" by itself, which becomes independent of the absolute physical location of the cell in the network. Stereotypy in time would then represent the signature of canonical operations specific to the binding topology of the activated graph, but not of the anatomical identity and location of the elements composing its nodes.

RELATIONSHIP BETWEEN UP AND DOWN CELLULAR STATES, TRANSIENT SYNCHRONIZATION OF ASSEMBLIES, AND GLOBAL DYNAMICS IN EEG MAPS

UP and DOWN States

A recent revival of attention has been accorded to membrane potential dynamics in cortical cells and the capacity of cells to engage in persistent activity for time periods up to a few seconds, compatible with the buildup of a working memory. If such behavior is well established in the striatum and prefrontal cortex, the presence of bistable units has been long disputed in primary and association cortex. UP states are not characterized by a specific pattern of persistent or synchronized activity; rather, they are associated with a high conductance state due to an intense afferent or recurrent synaptic bombardment. This ongoing bombardment sets the membrane potential of the target cell in a constantly depolarized state, just below the spike firing threshold. During this behavior, cortical and striatal cells exhibit bimodality in the distribution of their membrane potential values, defining two states: one in the vicinity of –70 to –80 mV (DOWN state) and one more depolarized by +15–20 mV (UP state). This behavior has been observed both *in vivo* and *in vitro*.

Although it has been strongly suggested that the tonic influence of attention-related and neuromodulatory signals, present in the awake and behaving animal, would force cortical cells to operate in the UP state most of the time (Stériade et al. 2001), most evidence for the description of bistable cells *in vivo* comes from the anesthetized preparation. Under xylazine and ketamine, spontaneous UP state periods last up from a hundred ms to up to several seconds. In slice preparations from ferrets, where the network is severely deafferented, the spontaneous occurrence of UP state episodes is rarely observed for normal artificial cerebral spinal fluid (ACSF) concentrations: the experimenter needs to promote the excitability of the cortical tissue by changing the potassium and calcium concentrations in order to reveal reproducible UP and DOWN transitions (Sanchez-Vives and McCormick 2000). However, in slices from mouse neocortex, UP and DOWN transitions are readily observed in normal ACSF.

The definition of UP and DOWN states, although based on the intracellular membrane potential dynamics of a single cell, may also be reflected in the level of recurrent activity in the local network, detectable by depth EEG recording (Paré et al. 1998). Indeed, positive EEG dips are the inverted image of the intracellular Vm behavior. Similarly, in the thalamus, the occurrence of UP states can be monitored by the detection of episodes of sustained burst multiunit activity. Divergent and recurrent connectivity leads to a strong self-modulatory influence of the cortex upon itself. An as yet unaddressed theoretical aspect is whether two-state dynamics are the only solution or whether the network state can wander across a larger but finite number of recurrence levels.

The part taken by intrinsic membrane properties and extrinsic drive, such as the balance between excitation and inhibition, remains to be clarified during UP states. In the pharmacologically activated cortical slice, McCormick's group finds an almost perfect balance between gI and gE during the UP state in prefrontal cortex. This voltage clamp-derived measure differs from theoretical estimates, based on ongoing activity in the primary visual cortex in the anaesthetized preparation ($gI = 4 - 6 \times gE$; Rudolph and Destexhe 2001; review in Destexhe et al. 2003), or from continuous conductance measurements done during visual activation (Monier et al. 2003). In the latter case, the UP state can also be evoked by nonoptimal stimuli, and the balance is often reached in terms of current and not conductance, clamping Vm just below spike initiation (for a theoretical prediction, see Shelley et al. 2002).

Is the UP State Instrumental in Building Up Synchrony?

What is the functional consequence of UP states in terms of spiking behavior and processing capacity of cortical neurons? On one hand, the ongoing bombardment due to massive recurrent activity during UP states may change the input–output transfer function of cortical neurons; this will be discussed in more depth later. On the other hand, because of the depolarized state, one may expect that synchrony in spike activity will be more easily detected when cells are

already in the UP state. This last question has been addressed both in the prefrontal cortex during the spontaneous generation of UP and DOWN states and in the primary visual cortex in response to a visual stimulus. Spike-triggered average records reveal that the UP states or visual responses are composed of two components: a large broad base of depolarization (which is termed "base" or "bias") and a 3–5 mV event lasting about 10–20 ms, which triggers the action potential. These results suggest that action potentials are triggered by the synchronous firing of a subset of presynaptic neurons (Nowak et al. 1997). More direct evidence has been obtained independently in single electrode voltage clamp, showing that for a specific set of stimulus features (velocity, direction, orientation), a light moving bar often evokes periodic bursts of excitatory inputs without or shifted in phase with inhibitory inputs. These packets of synchronous events last 10–20 ms and their phase onset can vary from trial to trial, suggestive of a reverberating process of intracortical origin (Bringuier et al. 1997).

Another set of observations, from McCormick's and Frégnac's laboratories, suggests that the inhibitory network is very important in the control of spike timing. In spontaneously active cortical networks, McCormick's study compares the IPSCs measured in voltage clamp of cortical pyramidal cells to 0 mV to EPSCs at –75 mV, and finds that IPSCs contain a much higher level of power at all frequencies above approximately 10 Hz (Hasenstaub et al. 2005). During visual stimulation, Frégnac's group examined the trial-by-trial frequency-time behavior of subthreshold membrane potential trajectories as a function of orientation and direction of the stimulus. High-frequency oscillatory behavior (40–90 Hz) is evoked during UP states in current clamp, while, in the same cell and under the same stimulus condition, the continuous voltage clamp measurement of excitatory and inhibitory conductances shows the presence of shunting inhibition (Monier et al. 2003; Russier et al. 2002).

One function of precise spike timing is to reduce the number of coactive afferents necessary to elicit a postsynaptic spike and increase selectivity of the association process: only closely spaced action potentials, emitted in the course of highly reproducible spike train patterns, will temporally summate and efficiently drive postsynaptic neurons. It is hypothesized that axoaxonic and basket GABAergic neurons may control precisely not only spike rate but also spike timing and thus may play an important role in both rate and time codes. Taking into account the fact that fast-spiking inhibitory interneurons are capable of transmitting higher-frequency information, the varying results summarized above suggest that high-frequency synchronized IPSPs are important for controlling rapid transitions in membrane potential and input conductance, leading to a high level of temporal precision in spiking behavior of pyramidal neurons.

Possible Functions for UP States

The functional role of the UP state remains open to conjecture. Are two states an epiphenomenon of network dynamics, a view shared by some of the participants

of our group, or are they a functional operating feature of the cerebral cortex, mediating working memory, attention, sensorimotor coordination, and other cortically generated computations? In support of the latter view, it has been proposed that a synchronous transition in the UP state may signal the general activation of a given microzone by a behavioral event and enable more easily the generation of action potentials (Stern et al. 1998). However, a review of the experimental evidence, partly unpublished, points to the diversity of effects of sensory stimulation on the dynamics of cortical UP and DOWN states. For instance, the sustained presentation of a full-field input (drifting grating) increases the UP state duration, preferentially in complex cells (Anderson et al. 2000). This effect appears specific to the neurons that share the same orientation preference. The authors suggest that UP states might participate in the cortical presentation of stimuli, or at least in stochastic resonance facilitating the integration of subthreshold inputs (see next section).

This interpretation differs from observations made in somatosensory cortex by correlating optical imaging and intracellular recordings. Subthreshold sensory synaptic responses evoked while a cortical area was engaged in an UP state were reported to be smaller in amplitude, shorter in duration, and spatially more confined (Petersen et al. 2003). These effects recorded at the single-cell level were correlated with local changes of cortical activity measured using optical imaging. The interpretation of these data, however, remains ambiguous since the lack of detectable change in the optical imaging signal—when the network is already in the UP state—is not surprising. A quantitative study of visual cortical receptive fields conditional to the membrane potential state occupied just before the arrival of the thalamic input shows on average a more neutral conclusion (Huguet et al. 2004). Two separate mechanisms of activation are revealed. The first is focal and transient and is linked specifically with sensory processing; when applying the appropriate reference statistics, subthreshold receptive fields are comparable in size when evoked from the DOWN state or the UP state. A more global activation process is triggered conjointly when one column in the cortical map switches from a DOWN state to an UP state. It corresponds to the slow lateral propagation of the UP state through horizontal connectivity and may be considered as nonspecific in terms of information processing. This viewpoint contradicts Petersen et al.'s (2003) conclusion; they interpret both activation processes as information specific and claim that both sensory-evoked PSPs and spiking are inhibited by spontaneously occurring UP states.

Functional Microstates in the Human Brain

Thus far, our discussion has been limited to the dynamics observed in the membrane potential of a single cell, or averaged across a cortical column extension, through optical recording with a spatial precision of 50–100 μm. Synchronicity and oscillatory behavior, however, is often more easily detected when averaging

over larger spatial scales and using macroprobes, such as local-field potential and EEG.

The term *functional microstate* is used to describe a particular, but very stable, empirical observation when recording multichannel human EEG (Lehmann et al. 1987). It is the observation that the spatial configuration of the global scalp electric field always shows stimulus-locked periods of stability separated by short transitions, which last on average around 80–120 ms. Based on this observation some authors have proposed that information processing is parsed into sequential episodes and that these episodes represent the basic building blocks underlying spontaneous or evoked information processing (Michel et al. 1999).

Distributed linear inverse solutions applied to these microstates show that each state is characterized by the activity of a distributed neuronal network implicating different areas of the brain. It is assumed that the duration of the segment corresponding to a fixed spatial pattern reflects the computation time that this particular network needs to accomplish a particular part of the task, that is, a step of information processing. The abrupt switch from one state to the other would be mainly due to the exclusion and inclusion of new modules in this network, leading to a dynamic relational reconfiguration of the large-scale cerebral neural network over time.

Methodologically, these functional microstates are confirmed when applying cluster analysis on the multichannel EEG data. It usually reveals that a limited set of electric field configurations (EEG maps) are sufficient to explain a given period of EEG activity (determined by cross-validation). Fitting these cluster maps to the data by spatial correlation analysis results in a discrete distribution of these maps, each one being present for a given duration. This procedure is independent of the strength of the activity since all maps are normalized to unitary strength. The cluster analysis thus only looks at the topography, the landscape of the scalp electric field. Nevertheless, segment borders typically (but not always) appear during low field strength, that is, during periods of low signal-to-noise ratio. It is not known whether these periods reflect low neuronal activity or highly nonsynchronized activity. Disease, drugs, external stimulation, or cognitive tasks can influence both the duration as well as the sequence of the microstate. Thus, the syntax with which these basic building blocks are put together may be crucial for the behavioral outcome.

Several questions were raised during the meeting, which remain to be solved:

- What characterizes the transition period? The duration of the switch from one microstate to the next may be affected by the algorithm used to detect sequential states. One should include in the search analysis the possibility that at certain times no stable EEG map is observed.
- Do the modules of the neural network during a microstate synchronize or not? If yes, in which frequency and is it phase-locked or not? Time-frequency analysis based on wavelets could be used to answer this question (Le Van Quyen et al. 2001).

- To what extent are spontaneous and evoked microstates comparable? A key issue is the possible "phase resetting" of ongoing EEG induced by the sensory stimulus, which may result in the presence of temporal segmentation into distinct microstates in the evoked case (Shah et al. 2004).
- How can the syntax of microstates be analyzed formally?
- Are microstates related to consciousness and do they support the workspace model introduced by Dehaene and Changeux (see below)?
- Can the UP and DOWN states observed at the cellular level participate in the buildup of these microstates? We note that UP and DOWN states have been observed mostly in the anesthetized and sleeping preparation, and that the eventual presence of synchronized depolarization spreading over large cortical areas should be detectable with the EEG.

NETWORK RECURRENCY, CORTICAL GAIN CONTROL, AND ATTENTIONAL PROCESSES

Attention and Sensory Processing

Because of its well-documented limitation in processing multiple tasks in parallel, the brain, and more specifically the cortex, has to find a way to select the relevant stimulus and allocate enough computing resources to the task. Attention during active behavior is a focalization process that seems critical for the detection of complex objects or even "pre-attentive" features of objects in low-level vision. Classically, a distinction is made between two forms: (a) *attentional selection* refers to the focus in attention targeted to an individual stimulus out of an array of competing stimuli, and (b) *attentional facilitation* refers to the performance increase in the detection of a single stimulus when it appears alone at an attended location. An electrophysiological correlate of this latter process has been found by the observation, in V4 of the macaque monkey, that behavioral attention directed to a location in the visual field increases the responsiveness of V4 cells to stimuli shown at that location (Reynolds et al. 2000). Responses to weaker stimuli are enhanced by attention, whereas those to stronger stimuli are unchanged. This observation provides an intriguing analogy with the possible role of ongoing synaptic intracortical bombardment and the local level of recurrence in the control of the cortical gain.

The results obtained in Reynolds's study favor the "contrast gain model," according to which the neuronal response is scaled as if the effective input stimulus strength had been increased multiplicatively by a constant factor. This corresponds to a lateral shift in the log-contrast-input–response function (transition from the dark gray (control) to the black curve in the left panel of Figure 19.4c). Evidence for such behavior pleads for a saturating or normalization mechanism in response strength, thus leading to a limitation in the ability to enhance output at high levels. Another model, supported by McAdams and Maunsell (1999) and

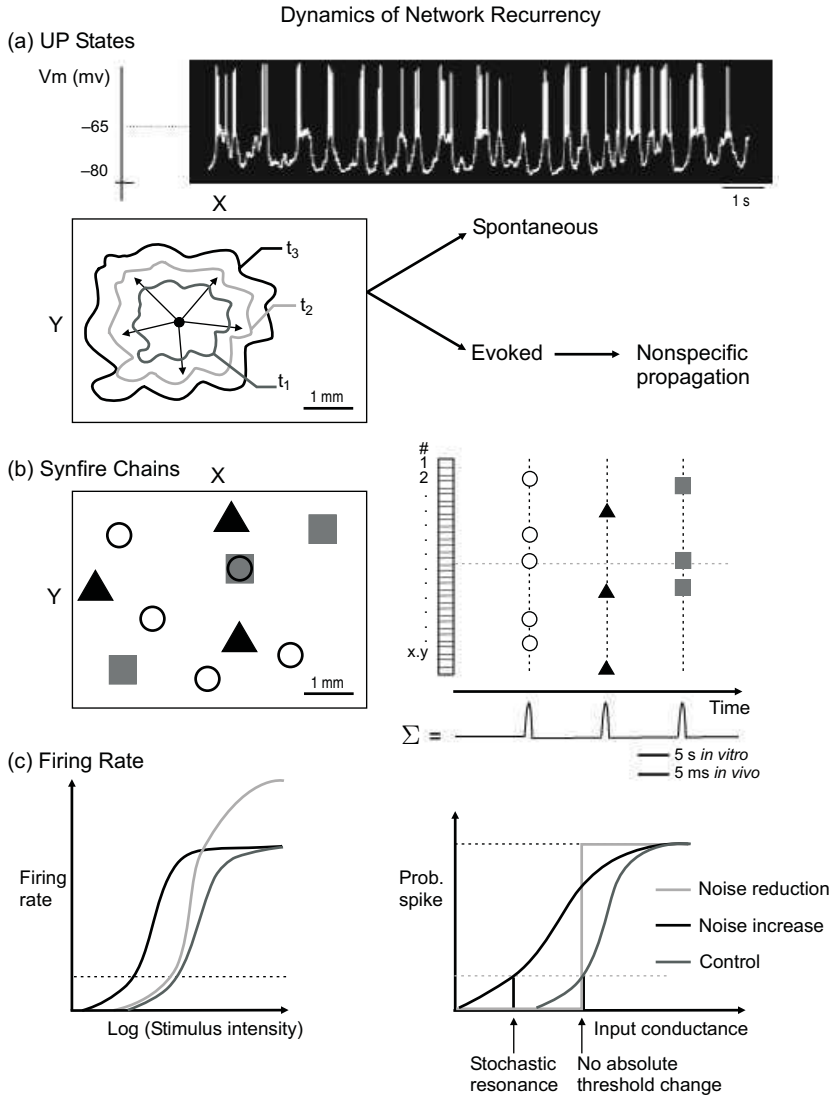

Figure 19.4 Recurrent networks = UP and DOWN states vs. synfire chains. (a) UP states: Recording of a V1 cell showing bistability in its membrane potential dynamics. The inset represents a spatial view of the laminar plane of cortex (X, Y) and the spatial spread of "UP"-state at different points in time (t_1, t_2, t_3). (b) Synfire chains. Left: sparse spatial distribution of cells belonging to the same synfire chain. Cells which are coactive during the same temporal window (defined on a 2 ms bin) are labeled by the same symbol. Right: raster view of the time course of the propagation of synchrony packets (symbols) across the cortical network (for N cells = x, y). $\sum$ = evolution with time of the total spike activity of the network (integrated over space). *Caption continues on next page*.

called "response gain model," proposes that attention causes a multiplicative increase in firing rate output (transition from the black (control) to the light gray curve in the left panel of Figure 19.4c). According to this model, it is the response neuronal firing rate that is multiplied by a constant gain factor.

A Cellular Analog of Attentional Facilitation

Background ongoing synaptic activity, misleadingly called "noise," emerges naturally in biological recurrent neural networks and can be recorded in the variance of membrane potential values in single cells in the awake or anesthetized animal (review in Destexhe et al. 2003). As seen earlier, the characteristics of the background activity vary with the level of alertness (sleep vs. awake and attentiveness) and also with the level and nature of anesthesia. The processing of input signals during sensory activation is not only affected by changes in the mean membrane potential (e.g., depolarization) due to the addition of a DC component but also by nonlinear effects due to the high-frequency content of the fluctuation spectrum as well as to changes in input conductance. The contextual impact of background activity on the probability of a spiking response to a test input has been studied intracellularly, both *in vivo*, when the membrane potential shifts to an UP state (see above), and in the *in vitro* situation under certain pharmacological conditions which facilitate the recruitment of reverberating intracortical activity and the generation of "UP-like states" (Shu et al. 2003a, b).

The following controversies remain:

- Most available experimental data show that the input–output probability curve measured in response to pulses of input conductance is smoothed and shifted towards weaker inputs compared to the control quiescent condition when the network is in the highly recurrent mode (high conductance state). Such input–output curve is used in sensory electrophysiology to measure the neurometric transfer function of the cell under study and can be compared to psychometric curves used in psychophysics, relating the

Figure 19.4 (*continued*) (c) Hypothetical modulatory effects of the network recurrence level on the transfer function of cortical neurons. The control input–output characteristics linking postsynaptic firing rate (left) or spiking probability (right) as a function of input strength are represented by dark gray curves. The "contrast gain model" (left panel, black curve) posits that the postsynaptic discharge rate is scaled as if the effective input stimulus strength had been increased multiplicatively by a constant factor. The "response gain model" proposes that attention causes a multiplicative increase (light gray curve) as if the response neuronal firing rate had been multiplied by a constant gain factor. Right: the equivalent effect found at the level of the probabilistic transmission by cortical neurons. If attention reduces the variance level, the threshold for spiking is unchanged but the input–output curve becomes a steep Heavyside function (Chance et al. 2002). If attention increases the variance level, the slope of the i/o curve is further smoothed and the threshold of significance for detecting weak input (dotted line) is improved (Destexhe et al. 2003).

percentage of correct choice with stimulus intensity. In the present case, the leftward shift of the neurometric curve (cf. the black [high conductance state] and dark gray [control quiescent state] curves in the right panel of Figure 19.4c) indicates that the detectability for weak inputs is increased by a lowering of the absolute spiking threshold (see arrows and dotted line in Figure 19.4c). This effect, reminiscent of stochastic resonance, is accompanied by a decrease of the slope of the neurometric function, suggestive of compensatory decrease in the cortical gain. This prediction was first formulated with theoretical models (Rudolph and Destexhe 2003).

- Another theoretical model attributes an opposite effect to attention-related processes by reducing, rather than increasing, noise variance (Chance et al. 2002). Background synaptic recurrent activity tunes the input–output gain of neurons and enhances the slope of the neurometric function without changing the absolute sensitivity threshold (light gray step-function curve in Figure 19.4c).

Whichever mechanism is put into play, both viewpoints predict an input rescaling, that is, a change in the dynamic range of input levels, which are coded in graded fashion by the output spiking probability. It remains to be established which effect is more likely in the behaving awake animal, and whether such gain control mechanisms at the neuronal level may be beneficial at the population coding level.

FROM CORTICAL SPACE TO DYNAMIC RECONFIGURATION OF PERCEPTUAL REPRESENTATIONS

The distribution of excitation in the cerebral cortex occurs in identified clusters of hot spots, suggestive of localized reverberation processes. It occurs locally, for example, through recurrent activation within the minicolumn, and clustering of activity is thought to propagate across the cortical network via patchy excitatory long-distance horizontal and cortico-cortical connections. Inhibition between minicolumns is mediated via different kinds of local inhibitory GABAergic interneurons, driven by afferent input as well as via synapses from local and distant pyramidal cells. Such anatomy of circuit architecture (Figure 19.5, top) is, of course, not specific to sensory and motor cortex. Similar layouts can be found, for instance, in the lamprey spinal locomotor CPG (see Kiehn et al., this volume). The basic building block here is an "excitatory core" of mutually excitatory premotor interneurons (EINs) connected by glutamate synapses having AMPA and NMDA receptors. Each hemisegment of the spinal cord has such a core, and long-distance reciprocal (glycinergic) inhibition between them secures left–right alternation, whose functional action is similar to long-range excitation of local inhibition in visual cortex. Two of the other motor systems

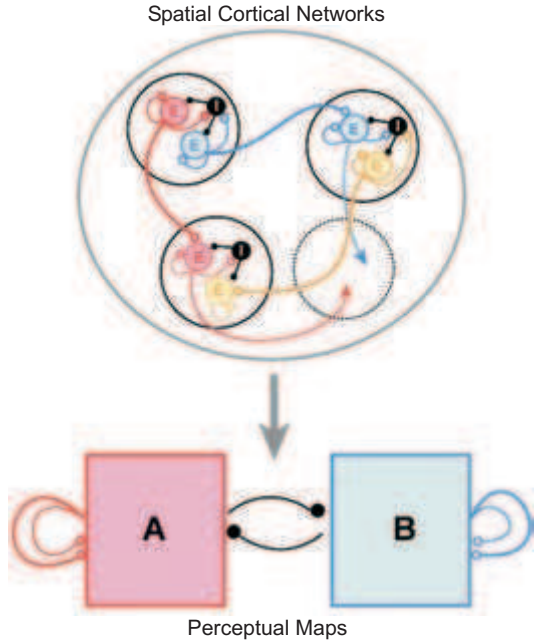

Figure 19.5 Building a perceptual space. Top: spatial schematic representation of activity in cortical networks. Cortical cells can be excitatory (E) or inhibitory (I) and situated in the same or different columns (circle). The same color for the E-cells represents coactive cells belonging to the same functional assembly. Bottom: representation of the dynamics between two color-coded assemblies in an abstract perceptual referential, through long- and short-distance excitatory and inhibitory connections. In addition to short-distance inhibitory connections, the model assumes the existence of a second type of local inhibitory interneuron (bipolar or double bouquet) that is driven mainly by long-range excitatory connections from pyramidal cells. These connections are important for achieving rhythmic activation of attractors and competition between them.

discussed during the workshop—the vertebrate respiratory oscillator in the brainstem and the saccade generator of the superior colliculus—also comprise an excitatory core. However, the precise composition and properties of these networks differ in important respects. For example, the respiratory excitatory core, the pre-Bötzinger complex, lacks NMDA transmission and relies on persistent sodium channels for burst buildup and plateau maintenance. The burst termination mechanism involves inactivation of the sodium-persistent I_{NaP} channels. The saccade generator of superior colliculus has a core of mutually exciting neurons in its deep layer. Here, AMPA and NMDA transmission is important for fast-burst initiation and to maintain a high-bursting frequency. The burst duration, however, is tightly controlled by externally provided disinhibition, possibly generated by long-distance connections recruiting the feedback action from the cerebellum.

Full-scale biophysical simulations (including compartmental cell models and AMPA and NMDA type synaptic transmission) in the lamprey locomotor CPG and a network model of layer II/III of visual cortex (Fransén and Lansner 1995 and unpublished observations) show clear similarities in their dynamic behavior. A way to give a possible functional relevance of this patchy pattern of excitation across the anatomical network is to project cells according to their degree of synchrony into an abstract "perceptual" space (bottom part of Figure 19.5). Elements of the cortical network synchronous at a given epoch in time (same-colored cells in the upper cartoon of Figure 19.5), which belong to the same or distinct "minicolumns" (circular clusters in Figure 19.5) and are synaptically connected, will define the Hebbian assembly participating in the broadcasting of an identified percept ("A" or "B" in the bottom part of Figure 19.5). If we assume the existence of several facilitated neural assemblies within the same network (color coded in Figure 19.5), they would compete in a kind of winner-take-all manner (as do the left–right sides in the spinal CPG). As one neural assembly wins and becomes active, it inhibits the others through local connections; its pyramidal cells are in an UP state but gradually hyperpolarize until activity terminates. This enables the emergence of some other neural assembly. Additional control will be imposed by afferent inputs that bias activation towards more stimulated assemblies. This analogy between neocortex and the spinal cord lends continuous dynamics to the attractor memory network paradigm (Yuste et al. 2005).

A PLAUSIBLE NEURAL ARCHITECTURE FOR THE EMERGENCE OF COGNITION AND ACCESS TO CONSCIOUSNESS

So far, only a few models have addressed the emergence of conscious cognitive processes in the human brain (review in Koch 2004). At this Dahlem Workshop, Jean-Pierre Changeux reviewed the basic concepts of hypothetical networks that could subserve the genesis of "higher-order" mental states and tried to relate predictions of the model with the observation of cellular UP states and synchrony microstates in the EEG (discussed above).

Selection of "Adequate" Actions and Decisions

The model, initially proposed by Dehaene and Changeux (1991; Dehaene, Dehaene-Lambertz, and Cohen 1998), assumes that the elementary mechanism of selection by a self-evaluated reward is restricted to a set of clusters of prefrontal neurons encoding for a repertoire of behavioral rules, the activation of which controls a lower-level sensorimotor network. Clusters are postulated to exhibit a high level of spontaneous activity together with strong recurrent

connectivity and thus to display two *stable* modes of activity: one in which the cluster is inactive, and the other similar to the UP state, in which activity, once initiated, remains at a high level for a prolonged period.

Action selection may be implemented by a stabilization–destabilization mechanism (see Dehaene and Changeux 1991). Negative reinforcement is assumed to cause a *fast synaptic desensitization* on a timescale of a few tens of milliseconds, which allows synapses to recover spontaneously their original strength on a slower timescale of a few seconds. The net result of this mechanism is that whenever negative reinforcement is received, recurrent connections within the currently active cluster decrease rapidly in strength, thus releasing the neighboring clusters from lateral inhibition. Spontaneous activity then propagates from one cluster to another, giving the full network/organism the chance to test different prerepresentations or behavioral options. Thus, reward signals function as effective *selection signals* and either maintain or suppress active prefrontal representations as a function of their current adequacy.

Distinction between Conscious and Nonconscious Processings

During sleep and deep general anesthesia, the subject is nonconscious with the notable, though limited, recall of the dreaming episodes. Even when awake, alert subjects may not be aware that they are carrying on intense *nonconscious* processing. Particular experimental evidence in favor of nonconscious processing (among many others) is the phenomenon of *semantic priming* (Dehaene, Naccache et al. 1998; review in Dehaene and Naccache 2001). Visual words, when flashed briefly before a masking stimulus, are not perceived consciously; that is, the subject/observer does not report their presence. Nevertheless, they still induce a priming effect since they facilitate the subsequent processing of related words by the same subject/observer. Brain imaging techniques reveal that such stimuli have a measurable influence on brain activation patterns, in particular in the areas involved in motor programming, which are covertly activated (Dehaene, Naccache et al. 1998). Moreover, these patterns differ dramatically when the subject (according to his/her reports) sees the stimulus in a fully conscious compared to a nonconscious manner.

The central proposition made by Changeux and Dehaene is the neural embodiment in the brain of two main distinct computational spaces. The first is a *processing network* composed of parallel, distributed, and functionally encapsulated processors organized from cortical *microcircuits.* These processors range from primary (or even heteromodal) sensory processors to motor processors and include long-term memory stores of semantic databases, the "self," autobiographical and personal data, as well as attentional and evaluative systems including motivation, reward, and, in general terms, the emotions. The second computational space, referred to as a *global workspace*, is assumed to consist of a distributed set of cortical neurons, which integrate physically the multiple

processors by their ability to receive from and send back long-range excitatory axons to homologous neurons dispersed in other cortical areas (Figure 17.1, this volume). These projections interconnect, at the brain scale, distant areas in the same hemisphere and between hemispheres through the *corpus callosum*. The early observations of Cajal (1909) and Von Economo (1929), which are supported by a large body of recent observations (see Mountcastle 1997), indicate that pyramidal cells from layers II and III of the cerebral cortex (among others) possess *long axonal processes* that they send within and between hemispheres. They are postulated to contribute to the neural workspace in a privileged manner. An important consequence of this postulate, already noticed by Von Economo (1929), is that pyramidal neurons of layers II and III are especially abundant in dorsal lateral prefrontal and inferoparietal cortical areas and thus offer, at the brain-scale level, a regional correlate of this cellular hypothesis in terms of a topology of activated cortical areas. As a result, these selective contributions might be directly evaluated by brain imaging techniques.

The model posits that, in a conscious effortful and attentive task, workspace neurons become spontaneously coactivated, forming discrete though variable spatiotemporal patterns of activity, giving rise to some kind of *global prerepresentation*. Such "brain-scale" representations would mobilize neurons from multiple brain processors in a reciprocal manner and be subject to regulation by vigilance and attention neuromodulators and to selection by reward signals. Their eventual recording as "functional microstates" in the EEG was discussed above.

WHAT SYSTEM NEUROSCIENCE CAN LEARN FROM INTERDISCIPLINARITY

A Need for New Paradigms for the Study of Brain Computation

Our understanding of brain complexity is often limited by the lack of a theoretical framework specifically adapted to the neural embodiment of computation, and experimenters often address key issues on the basis of anecdotal (since devoid of context) observations or intuitive speculations. It is desirable for theory and modeling in neuroscience to be carried out in a dedicated and systematic manner rather than in an ad hoc fashion, taking into account available information and know-how from other relevant disciplines, such as computer science, robotics, and mathematics. Such interdisciplinary pooling of information is especially needed for a transition from currently existing descriptive models for computation in neural microcircuits (that may, for example, describe activity streams resulting from a particular input) to functional models that can—on the basis of general principles—guarantee that the circuit carries out a desirable computational task for *all* (in general, exponentially many) possible inputs. For example:

- To make educated guesses about which computational problems are solved by the brain (e.g., by mice), one can learn quite a bit from the real-time computing problems regarding motor control and processing of complex sensory input streams that are encountered in robotics. The difficulty of such computational tasks is easily underestimated because evolution has solved these problems so well, and the complexity of the underlying computation problems are hidden from us.
- To evaluate the power of computational problems, one needs to take into account results from computational complexity theory.
- To evaluate the difficulty of learning-specific problems (and the difficulty of generalizing learned knowledge), one needs to take into account results from statistical learning theory and empirical results from machine learning.
- To design new tests for analyzing the structure and progress of computations in a neural system (besides looking for neurons whose response in a trained animal can be related directly to its behavior), one needs to take into account methods from system identification and dynamical systems theory. Typically, these methods require probing the response of the system (or parts of the system) for a much richer ensemble of quasi natural stimuli (with the same statistics and addressing the full real-time parallel processing capacity of the sensory cortical analyzer).

Reviving Liquid Computing

High-level cognitive functions in the human brain involve the activation of processes (from the activation of the ACh receptor to memory) with time constants covering 13 orders of magnitude (from microseconds to years). These microcircuits implement massively parallel computations, where the inputs consist of multimodal input streams stemming from a rapidly changing environment, and results of computations have to be provided at any time. In contrast, nearly all of the previous theoretical approaches to understand how such systems can possibly provide the basis for cognition and learning (e.g., Dayan and Abbott 2001) have been based on highly simplified and homogenized neurons and synapses as well as on simplified connection patterns, and they result in simple dynamics converging towards a set of point attractors. New paradigms of computation that depart significantly from this concept are needed, where the complexity expressed in the recurrent ongoing activity of the network is the determining part of the computational process rather than something to be avoided.

Over several decades, a number of theoretical frameworks have been proposed to understand how collective and complex behavior of large populations of units may lead to a distributed representation of information (Longuet-Higgins 1968; Gabor 1969; Kohonen 1977; Hinton and Anderson 1981; Hopfield 1982). These now classic "holographic" theories have recently been the subject

of renewed interest addressing the framework of complex dynamical systems, such as the "dynamics-based computing" (Sinha and Ditto 1998), computing using neuronal diversity (Buonomano and Merzenich 1995), or the "liquid computing" (Maass et al. 2002) paradigms. The goal of this latter approach is to provide a conceptual framework for analyzing emerging real-time computing capabilities of neural microcircuits and to produce better quantitative methods for testing computational capabilities of neural microcircuits *in vitro* and *in vivo*.

Theoretical predictions and computer simulations suggest that under certain conditions the current state of a dynamical system contains information about current and recent inputs injected into the system, and, furthermore, that even the results of nonlinear computations on the components of this input can be read out and extracted by linear methods (i.e., by a weighted sum, or by a perceptron in the case of a classification task) from the current state of such a dynamical system. One condition for this is that the dynamical system contains sufficiently diverse components (e.g., as provided by a rich repertoire of different types of neuronal time constants and synaptic kinetics). Another condition is that the circuit has a sufficiently complex connectivity structure (containing connections to nearest neighbors as well as a distribution of midrange connections). Hence, many dynamical systems constructed by theoreticians (which often consist of stereotypical simple components and all-to-all or only-to- neighbors connections) do not satisfy these conditions, but reasonably realistic models for neural microcircuits tend to satisfy these conditions.

Computer simulations show that an optimization of the computational power of such neural microcircuit models requires that these circuits are sufficiently activated by the input stream. An idea that emerged from this Dahlem Workshop, which has been already tested on computer models, is that UP states of neural microcircuits constitute activity regimes that enhance specifically their capability for fast nonlinear computations on time-varying firing rates (Maass et al. 2005). Another topic of current research is the impact of readout neurons, which extract information from such microcircuits and project their output back into the circuit. It can be shown that they create high-dimensional attractors of the circuit state that provide longer memory spans for selected input patterns (Maass et al. 2006). This still leaves other components of the circuit state free for online processing of new information. This approach using realistic neural microcircuit models suggests new ways of understanding emergent computations while processing continuously different input streams. It underlines the role of hierarchies of cortical areas where higher-level circuits are optimized for processing on a slower timescale the output streams provided by neural readouts from lower-level circuits.

Evolutionary Considerations Using Language as an Example

Evolution by natural selection is the only known force to build up complex adaptations. Hence it makes sense to think that the nervous system is also the result

of evolution, and that it fulfills many different roles. This is not to say, however, that every aspect of it is selectively significant, let alone perfect. Evolutionary optima do not necessarily coincide with engineering ones for at least three reasons: (a) there are several entrenched state characters, so-called hang-ups from former evolution; (b) the available genetic variation may be limited (so-called constraints), and (c) in the process of selection, only relative (rather than absolute) fitness matters. Thus, having a modern mammalian neocortex may render the hippocampus obsolete from an engineering point of view, but evolution cannot go back to the drawing board and start all over again. Evolution is a process of "irrational design" (Sydney Brenner), often reminiscent of tinkering (*bricolage,* François Jacob). It is not yet known to what extent various aspects of the nervous system are selectively neutral or maladaptive from an engineering point of view.

Cumulative selection can produce complex adaptations, as Darwin and Wallace already recognized, but this is not always obvious. In the case of the optical structure of the eye, Darwin had no choice but to assume that natural selection would have driven the populations through a series of incremental improvements, acting on appropriate genetic variation (Darwin 1859). Nilsson and Pelger (1994) did demonstrate about 130 years later that Darwin was essentially correct. They applied computer simulations to generate eyes, and selection was based on the quality of the imaging capacity of the structure. Fortunately, biophysics is advanced enough to make this calculation, which was then used later to calculate the relative fitness of each eye. It turned out that starting with only three cell layers (a transparent, a pigment, and a sensitive layer), they could reconstruct how the eye of, say, a fish could have arisen in a surprisingly small time in evolution. Of course, they did not consider the visual analyzer, but this had not been their task in the first place.

A relevant question is, therefore, to ask how the neural structures fulfilling certain (occasionally quite complicated) styles could have evolved. Ultimately, one would like to simulate such evolution. Leigh Van Valen said that evolution was the control of development by ecology. In a less telegraphic form, one can imagine the following algorithmic process (see Figure 19.6). What are the relevant traits for a simulated evolution of nervous systems? An essential ingredient is the genetic determination of neuronal types by the following traits: number of

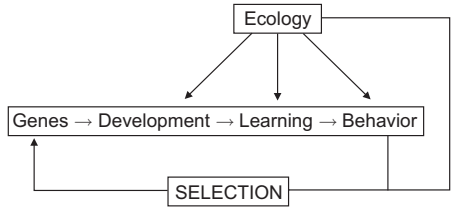

Figure 19.6 Schematic depicting the control of development by ecology.

types, number of cells in each type, cell shape, projection pattern, synaptic rules, and electric properties. One has to start with a simple architecture and to see the evolution of a network that shows a required behavior as a result of selection on the population of networks. This approach is not entirely novel: Floreano has used it to generate control networks for robots (Floreano and Urzelai 2000), and Rolls and Stringer (2001) evolved an autoassociative network. However, one should attempt more complex networks as well.

The issue of the origin of natural language constitutes an appropriate field of application of such theories to the emergence of higher cognitive functions. For example, Fitch and Hauser (2004) have shown that tamarind monkeys are, in contrast to young human infants, insensitive to violation of input described by phrase structure grammar $(AB)^n$, whereas they are proficient at discerning patterns from finite state grammar A^nB^n. What could be the neuronal background to this important difference? At present, we do not know, but we might learn it through simulated evolution of neural architectures.

Finally, an interesting mechanism, likely to have played a role in the evolution of language, is worth mentioning; namely, genetic assimilation (or Baldwin effect). In our context it is a mechanism whereby learning can guide evolution, rather than vice versa. Imagine a flat fitness landscape with a needle-like peak somewhere. There is no way genetic evolution can find the needle, unless the individuals are plastic (in the sense of genetics—this includes learning as well). Plastic individuals have a fair chance to "sense" the needle through their phenotypic range and thus be subject to directional selection towards it. It is interesting to note, however, that plasticity becomes maladaptive as soon as the genotypes come sufficiently close to the needle: plasticity is costly then without advantage. Then the trait is fully genetically assimilated (it was once learned; now it is fixed). For complex traits evolving on complex fitness landscapes, one dimension may favor the loss of plasticity whereas in another, learning might always be the best. In the case of language, vocabulary is stored in the environment and is subject to rapid change. In contrast, certain procedural operations behind syntax are likely to be innate. How did they evolve? One mechanism may be genetic assimilation, which could have led to an appropriate fixation of certain rather widespread network properties of the human brain. Regulatory genes acting on neurons are likely to have been involved.

CONCLUSION

Although some stereotypy was found in the molecular determinants and anatomical substrate of cortical circuits, it is likely that the expression of cortical computation cannot be easily decomposed in stable—time-invariant and space-invariant—modules. We propose that the modularity lies not in the structural arrangement but in the dynamical signature of the activity that circuits sustain. Cortical computation, and the emergence of cognitive functions, should be

envisioned as a dynamic coordination of reconfigurable modules coexisting within a finite number of regular structural architectures.

A central theme of our discussions was to clarify distinct scales in the spatial and temporal selectivity of synchrony. Recently, some confusion seems to have arisen between synfire chains, hot spots of synchronized activity, and UP states. The conclusion reached at this Dahlem Workshop is that these different synchronization processes differ significantly in terms of spatial spread in the cortical tissue, propagation mode, and propagation speed:

- Synfire chains are the sparse version of an assembly, where the reproducibility of temporal motifs is in the millisecond range and is found in the phase relationships between activities of individual cells. The highly reproducible intervals can last up to several hundred of milliseconds, irrespectively of the spatial separation of the cortical units. It is best described by a reverberation of activity in a local network where the same cell can participate several times in the propagation of the same synfire chain.
- Bistability in the membrane potential is often observed in cortical cells, both *in vitro* and *in vivo*. UP states are usually triggered by intense feedforward activity. Their initial spatial spread is compact, and the local initiation of an UP state gives rise to a slow propagating wave of depolarization in the surrounding cortical tissue, most likely through horizontal connectivity. Their occurrence cannot be considered as signaling a bursty event of spike synchrony at a given node of the network, since they characterize more a membrane potential state defined with a temporal precision in the second range than a specific pattern of spiking activity.
- A mixed version of synfire chains and UP states has been revealed recently using spike or membrane potential recordings paired with calcium imaging. The spatial spread that results from this process is locally compact, with additional hot spots that are sparsely distributed across the network. The propagation of activity jumps from one spot to the next and transitions dates form temporal motifs similar to those observed in synfire chains.

In summary, "synfire chains" are defined by phased-locked activity between few members of the cortical assembly, forming temporal motifs with a precision in the order of milliseconds. UP states are defined by the observation of correlated or synchronous depolarization in the membrane potential of cortical cells clustered in a restricted cortical neighborhood. Chains of propagation of UP states can be viewed as the analog version of synfire chains when one spike is replaced by an UP state transition of cortical columns in a high conductance mode favoring spike activity. These transitions, occurring at different times in distant spatial locations of the network, would shape the temporal sterotypy of the cortical song.

UP states may prove to be an epiphenomenon of recurrent networks. However, the possibility exists that they are essential to the binding of sensory

information: they may be the microcircuit substrate underlying what Yves Delage has described as the "parasynchronization" process necessary for the emergence of mental imagery and dreams (Delage 1919).

REFERENCES

Abeles, M. 1982. Local Cortical Circuits. An Electrophysiological Study. New York: Springer.

Abeles, M. 1991. Corticonics: Neuronal Circuits of the Cerebral Cortex. Cambridge: Cambridge Univ. Press.

Abeles, M., and G.L. Gerstein. 1988. Detecting spatiotemporal firing patterns among simultaneously recorded single neurones. *J. Neurophysiol.* **60**:909–924.

Ahmed, B., J.C. Anderson, R.J. Douglas, K.A.C. Martin, and J.C. Nelson. 1994. Polyneuronal innervation of spiny stellate neurons in cat visual cortex. *J. Comp. Neurol.* **341**:39–49.

Albus, K. 1975. A quantitative study of the projection area of the central and the paracentral visual field in area 17 of the cat. II. The spatial organization of the orientation domain. *Exp. Brain Res.* **24**:181–202.

Anderson, J., I. Lampl, I. Reichova, M. Carandini, and D. Ferster. 2000. Stimulus dependence of two-state fluctuations of membrane potential in cat visual cortex. *Nat. Neurosci*. **3**:617–21.

Arieli, A., A. Sterkin, A. Grinvald, and A. Aertsen. 1996. Dynamics of ongoing activity: Explanation of the large variability in evoked cortical responses. *Science* **273**: 1868–1871.

Barlow, H.B. 1972. Single units and sensation: A neurone doctrine for perceptual psychology? *Perception* **1**:371–394.

Basole A., L.E. White, and D. Fitzpatrick. 2003. Mapping multiple features in the population response of visual cortex. *Nature* **423**:986–990.

Bienenstock, E. 1996. On the dimensionality of cortical graphs. *J. Physiol. Paris* **90**:251–256.

Bienenstock, E., and R. Doursat. 1991. Issues of representation in neural networks. In: Representations of Vision, ed. A. Gorea, Y. Frégnac, Z. Kapoula, and J. Findlay, pp. 47–67. Cambridge: Cambridge Univ. Press.

Binzegger, T., R.J. Douglas, and K.A.C. Martin. 2004. A quantitative map of the circuit of cat primary visual cortex. *J. Neurosci.* **24**:8441–8453.

Blatow, M., A. Rozov, I. Katona et al. 2003. A novel network of multipolar bursting interneurons generates theta-frequency oscillations in neocortex. *Neuron* **38**:805–817.

Bok, S.T. 1936. A quantitative analysis of the structure of the cerebral cortex. *Proc. Acad. Sci. Amst.* **35**:1–55.

Bringuier, V., F. Chavane, L. Glaeser, and Y. Frégnac. 1999. Horizontal propagation of visual activity in the synaptic integration field of area 17 neurons. *Science* **283**: 695–699.

Bringuier, V., Y. Frégnac, A. Baranyi, D. Debanne, and D. Shulz. 1997. Synaptic origin and stimulus dependency of neuronal oscillatory activity in the primary visual cortex of the cat. *J. Physiol.* **500**:751–774.

Buonomano, D.V., and M.M. Merzenich. 1995. Temporal information transformed into a spatial code by a neural network with realistic properties. *Science* **267**:1028–1030.

Cajal, S.R. 1909. Histologie du Système Nerveux de l'Homme et des Vertébrés. Paris: Maloine.

Cavanagh, P. 2003. The language of vision. The Perception 2003 Lecture. ECVP, Paris, September. *Perception* **32**:1.

Chance, F.S., L.F. Abbott, and A.D. Reyes. 2002. Gain modulation from background synaptic input. *Neuron* **35**:773–782.

Chklovskii, D.B., T. Schikorski, and C.F. Stevens. 2002. Wiring optimization in cortical circuits. *Neuron* **34**:341–347.

Darwin, C. 1859. On the Origin of Species.

Dayan, P., and L.F. Abbott, eds. 2001. Theoretical Neuroscience: Computational and Mathematical Modeling of Neural Systems. Cambridge, MA: MIT Press.

DeFelipe, J., L. Alonso-Nanclares, and J.I. Arellano. 2002. Microstructure of the neocortex: Comparative aspects. *J. Neurocytol.* **31**:299–316.

Dehaene, S., and J.-P. Changeux. 1991. The Wisconsin card sorting test: Theoretical analysis and modeling in a neuronal network. *Cereb. Cortex* **1**:62–79.

Dehaene, S., G. Dehaene-Lambertz, and L. Cohen. 1998. Abstract representations of numbers in the animal and human brain. *TINS* **21**:355–361.

Dehaene, S., and L. Naccache. 2001. Towards a cognitive neuroscience of consciousness: Basic evidence and a workspace framework. *Cognition* **79**:1–37.

Dehaene, S., L. Naccache, H.G. Le Clec et al. 1998. Imaging unconscious semantic priming. *Nature* **395**:597–600.

Delage, Y. 1919. Le Rève: Etude Psychologique, Philosophique et Litteraire. Paris: Presses Universitaires de France.

Destexhe, A., M. Rudolph, and D. Paré. 2003. The high-conductance state of neocortical neurons *in vivo*. *Nat. Rev. Neurosci*. **4**:739–751.

Douglas, R.J., and K.A.C. Martin. 1991. A functional microcircuit for cat visual cortex. *J. Physiol*. **440**:735–769.

Douglas, R.J., and K.A.C. Martin. 2004. Neuronal circuits of the neocortex. *Ann. Rev. Neurosci*. **27**:419–51.

Douglas, R.J., K.A.C. Martin, and D. Witteridge. 1989. A canonical microcircuit for neocortex. *Neural Comput.* **1**:480–488.

Elston, G.N., R. Benavides-Piccione, and J. DeFelipe. 2001. The pyramidal cell in cognition: A comparative study in human and monkey. *J. Neurosci*. **21**:RC163.

Elston, G.N., and J. DeFelipe. 2002. Spine distribution in cortical pyramidal cells: A common organizational principle across species. *Prog. Brain Res*. **136**:109–133.

Fitch, W.T., and M.D. Hauser. 2004. Computational constraints on syntactic processing in a nonhuman primate. *Science* **303**:377–380.

Fitzpatrick, D. 1996. The functional organization of local circuits in visual cortex: Insights from the study of tree shrew striate cortex. *Cereb. Cortex* **6**:329–341.

Fleischhauer, K. 1978. The tangential organization of the cat motor cortex. *Verh. Anat. Ges.* **72**:725–726.

Floreano, D., and J. Urzelai. 2000. Evolutionary robots with on-line self-organization and behavioral fitness. *Neural Networks* **13**:431–443.

Fransén, E., and A. Lansner. 1995. Low spiking rates in a population of mutually exciting pyramidal cells. *Network Comput. Neural Syst.* **6**:271–288.

Frégnac, Y., P. Baudot, M. Levy, and O. Marre. 2005. An intracellular view of time coding and sparseness of cortical representation in V1 neurons during virtual oculomotor exploration of natural scenes. In: Cosyne (http://www.cosyne.org/program05/main.html).

Frégnac, Y., and V. Bringuier. 1996. Spatio-temporal dynamics of synaptic integration in cat visual cortical receptive fields. In: Brain Theory: Biological Basis and Computational Theory of Vision, ed. A. Aertsen and V. Braitenberg, pp. 143–199. Amsterdam: Springer.

Frégnac, Y., C. Monier, F. Chavane, P. Baudot, and L. Graham. 2003. Shunting inhibition, a silent step in visual cortical computation. *J. Physiol.* **97**:441–451.

Gabor, D. 1969. Associative holographic memories. *IBM J. Res. Devel.* **13**:156–159.

Galarreta, M., and S. Hestrin. 2001. Electrical synapses between GABA-releasing interneurons. *Nat. Rev. Neurosci.* **2**:425–433.

Georgopoulos, A.P., A.B.Schwartz, and R.E. Kettner. 1986. Neuronal population coding of movement direction. *Science* **233**:1416–1419.

Gilbert, C.D., and T.N. Wiesel. 1979. Morphology and intracortical projections of functionally characterized neurones in the cat visual cortex. *Nature* **280**:120–125.

Gilbert, C.D., and T.N. Wiesel. 1983. Clustered intrinsic connections in cat visual cortex. *J. Neurosci.* **3**:1116–1133.

Grinvald, A., E.E. Lieke, R.D. Frostig, and R. Hildesheim. 1994. Cortical point-spread function and long-range lateral interactions revealed by real-time optical imaging of macaque monkey primary visual cortex. *J. Neurosci*. **14**:2545–2568.

Gupta, A., Y. Wang, and H. Markram. 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. *Science* **287**:273–278.

Hasenstaub, A., Y. Shu, B. Haider et al. 2005. Inhibitory postsynaptic potentials carry synchronized frequency information in active cortical networks. *Neuron* **47**:423–435.

Hinton, G.E., and J.A. Anderson. 1981. Parallel Models of Associative Memory. Hillsdale, NJ: Erlbaum.

Hopfield, J.J. 1982. Neural networks and physical systems with emergent collective computational abilities. *PNAS* **79**:2554–2558.

Hubel, D.H., and T.N. Wiesel. 1962. Receptive fields, binocular interaction, and functional architecture in the cat's visual cortex. *J. Physiol.* **160**:106–154.

Hubel, D.H., and T.N. Wiesel. 1963. Shape and arrangements of columns in the cat's striate cortex. *J. Physiol.* **165**:559–568.

Hubel, D.H., and T.N. Wiesel. 1974. Uniformity of monkey striate cortex: A parallel relationship between field size, scatter, and magnification factor. *J. Comp. Neurol.* **158**:295–306.

Hubel, D.H., and T.N. Wiesel. 1977. Functional architecture of macaque monkey visual cortex. *Proc. R. Soc. Lond. B* **198**:1–59.

Huguet, N., G. Sadoc, P. Baudot et al. 2004. Conditional maps of visual cortical receptive fields: Up- and Down- state dependency in visually evoked dynamics. *Neuroscience* **34**:648.

Ikegaya, Y., G. Aaron, R. Cossart et al. 2004. Synfire chains and cortical songs: Temporal modules of cortical activity. *Science* **304**:559–564.

James, W. 1890. Psychology: Briefer Course. Cambridge, MA: Harvard Univ. Press.

Koch, C. 2004. The Quest for Consciousness: A Neurobiological Approach. Greenwood Village, CO: Roberts & Co.

Kohonen, T. 1977. Associative Memory: A System Theoretical Approach. Berlin: Springer.

Lehmann, D. 1971. Multichannel topography of human alpha EEG fields. *Electroenceph. Clin. Neurophysiol.* **31**:439–449.

Lehmann, D., H. Ozaki, and I. Pal. 1987. EEG alpha map series: Brain micro-states by space-oriented adaptive segmentation. *Electroenceph. Clin. Neurophysiol.* **67**:271–288.

Le Van Quyen, M., J. Martinerie, V. Navarro, M. Baulac, and F.J. Varela. 2001. Characterizing neurodynamic changes before seizures. *J. Clin. Neurophysiol.* **18**:191–208.

Longuet-Higgins, H.C. 1968. The non-local storage of temporal information. *Proc. R. Soc. Lond. B* **171**:327–334.

Lorente de Nó, R. 1938. Architectonics and structure of the cerebral cortex. In: Physiology of the Nervous System, ed. J.F. Fulton, pp. 291–330. New York: Oxford Univ. Press.

Maas, W., P. Joshi, and E.D. Sontag. 2006. Principles of real-time computing with feedback applied to cortical microcircuit models. In: Advances in Neural Information Processing Systems, vol. 18. Cambridge, MA: MIT Press, in press.

Maass, W., R. Legenstein, and N. Bertschinger. 2005. Methods for estimating the computational power and generalization capability of neural microcircuits. In: Advances in Neural Information Processing Systems, vol 17, ed. L.K. Saul, Y. Weiss, and L. Bottou, pp. 865–872. Cambridge, MA: MIT Press. Available online as #160 from http://www.igi.tugraz.at/maass/ publications.html

Maass, W., T. Natschlager, and H. Markram. 2002. Real-time computing without stable states: A new framework for neural computation based on perturbations. *Neural Comput.* **14**:2531–2560.

Malach, R. 1992. Dendritic sampling across processing streams in monkey striate cortex. *J. Comp. Neurol*. **315**:303–312.

Markram, H., J. Lübke, M. Frotscher, and B. Sakmann. 1997. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**:213–215.

McAdams, C.J., and J.H.R. Maunsell. 1999. Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. *J. Neurosci.* **19**:431–441.

Mead, C., ed. 1989. Analog VLSI and Neural System. Reading, MA: Addison-Wesley.

Mel, B.W. 2003. Why have dendrites? A computational perspective. In: Dendrites, ed. G. Stuart, N. Spruston, and M. Hausser, pp. 271–289. Oxford: Oxford Univ. Press**.**

Michel, C.M., M. Seeck, and T. Landis. 1999. Spatiotemporal dynamics of human cognition. *News Physiol. Sci.* **14**:206–214.

Mitchison, G. 1991. Neuronal branching patterns and the economy of cortical wiring. *Proc. R. Soc. Lond. B* **245**:151–158.

Monier, C., F. Chavane, P. Baudot, L. Graham, and Y. Frégnac. 2003. Orientation and direction selectivity of excitatory and inhibitory inputs in visual cortical neurons: A diversity of combinations produces spike tuning. *Neuron* **37**:663–680.

Monyer, H., and H. Markram. 2004. Interneuron diversity series: Molecular and genetic tools to study GABAergic interneuron diversity and function. *TINS* **27**:90–97.

Mooser, F., W.H. Bosking, and D. Fitzpatrick. 2004. A morphological basis for orientation tuning in primary visual cortex. *Nat. Neurosci*. **78**:872–879.

Mountcastle, V.B. 1997. The columnar organization of the neocortex. *Brain* **120 Pt. 4**:701–722.

Mountcastle, V.B., and G.M. Edelman, eds. 1982. Mindful Brain: Cortical Organization and the Group-selective Theory of Higher Brain Function. Cambridge, MA: MIT Press.

Nilsson, D.-E., and S. Pelger. 1994. A pessimistic estimate of the time required for an eye to evolve. *Science* **256**:53–58.

Nowak, J.Z., J.B. Zawilska, and K. Trzepizur. 1997. Alpha 2-adrenergic receptors regulate generation of cyclic AMP in the pineal gland, but not in cerebral cortex of chick. *Pol. J. Pharmacol.* **49**:137–141.

Paré, D., E. Shink, H. Gaudreau, A. Destexhe, and E. Lang. 1998. Impact of spontaneous synaptic activity on the resting properties of cat neocortical pyramidal neurons *in vivo*. *J. Neurophysiol.* **79**:1450–1460.

Peters, A., and I.R. Kaiserman-Abramof. 1970. The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites, and spines. *Am. J. Anat.* **127**:321–355.

Peters, A., and T.M. Walsh. 1972. A study of the organization of apical dendrites in the somatic sensory cortex of the rat. *J. Comp. Neurol.* **144**:253–268.

Petersen, C.C., T.T. Hahn, M. Mehta, A. Grinvald, and B. Sakmann. 2003. Interaction of sensory responses with spontaneous depolarization in layer II/III barrel cortex. *PNAS* **100**:13,638–13,643.

Rakic, P. 1988. Specification of cerebral cortical areas. *Science* **241**:170–176.

Reinagel, P., and R.C. Reid. 2000. Temporal coding of visual information in the thalamus. *J. Neurosci.* **20**:5392–5400.

Reynolds, J. H., T. Pasternak, and R. Desimone. 2000. Attention increases sensitivity of V4 neurons. *Neuron* **26**:703–714.

Riehle, A., S. Grün, M. Diesmann, and A. Aertsen. 1997. Spike synchronization and rate modulation differentially involved in motor cortical function. *Science* **278**:1950–1953.

Rieke, F. 1999. Computing with lipids, proteins, and ions. *Neuron* **23**:31–32.

Rolls, E.T., and S.M. Stringer. 2001. A model of the interaction between mood and memory. *Network Comput. Neural Syst.* **12**:89–109.

Rudolph, M., and A. Destexhe. 2001. Do neocortical pyramidal neurons display stochastic resonance? *J. Comp. Neurosci.* **11**:19–42.

Russier, M., I.L. Kopysova. N. Ankri, N. Ferrand, and D. Debanne. 2002. GABA and glycine co-release optimizes functional inhibition in rat brainstem motoneurons *in vitro*. *J. Physiol*. **541(1)**:123–137

Sanchez-Vives, M.V., and D.A. McCormick. 2000. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat. Neurosci.* **3**:1027–1034.

Schmidt, K.E., R. Goebel, S. Lowel, and W. Singer. 1997. The perceptual grouping criterion of colinearity is reflected by anisotropies of connections in the primary visual cortex. *Eur. J. Neurosci.* **9**:1083–1089.

Schummers, J., J. Marino, and M. Sur. 2002. Synaptic integration by V1 neurons depends on location within the orientation map. *Neuron* **36**:969–978.

Shah, A.S., S.L. Bressler, K.H. Knuth et al. 2004. Neural dynamics and the fundamental mechanisms of event-related brain potentials. *Cereb. Cortex* **14**:476–483.

Shapley, R., M. Hawken, and D.L. Ringach. 2003. Dynamics of orientation selectivity in the primary visual cortex and the importance of cortical inhibition. *Neuron* **38**: 689–699.

Sharma, J., A. Angelucci, and M. Sur. 2000. Induction of visual orientation modules in auditory cortex. *Nature* **404**:841–847.

Sharon, D., and A. Grinvald. 2002. Dynamics and constancy in cortical spatiotemporal patterns of orientation processing. *Science* **295**:512–515.

Shelley, M., D. McLaughlin, R. Shapley, and J. Wielaard. 2002. States of high conductance in a large-scale model of the visual cortex. *J. Comput. Neurosci.* **13**:93–109.

Sholl, D.A. 1956. The Organization of the Cerebral Cortex. London: Methuen.

Shu, Y., A.R. Hasenstaub, M. Badoual, T. Bal, and D.A. McCormick. 2003a. Barrage of synaptic activity control the gain and sensitivity of cortical neurons. *J. Neurosci.* **23**:10,388–10,401.

Shu, Y., A. Hasenstaub, and D.A. McCormick. 2003b. Turning on and off recurrent balanced cortical activity. *Nature* **423**:288–293.

Sinha, S., and W.L. Ditto. 1998. Dynamics based computation. *Phys. Rev. Lett.* **81**: 2156–2159.

Somers, D.C., E.V. Todorov, A.G. Siapas et al. 1998. A local circuit approach to understanding integration of long-range inputs in primary visual cortex. *Cereb. Cortex* **8**:204–217.

Spurzheim, G. 1824. The Anatomy of the Brain, with a General View of the Nervous System. London: S. Highley.

Stern, E. A., D. Jaeger, and C.J. Wilson. 1998. Membrane potential synchrony of simultaneously recorded striatal spiny neurons *in vivo*. *Nature* **394**:475–478.

Swindale, N.V. 1981. Patches in monkey visual cortex. *Nature* **293**:509–510.

Toledo-Rodriguez, M., B. Blumenfeld, C. Wu et al. 2004. Correlation maps allow neuronal electrical properties to be predicted from single-cell gene expression profiles in rat neocortex. *Cereb. Cortex* **14**:1310–1327.

Trachtenberg, J.T., and M.P. Stryker. 2001. Rapid anatomical plasticity of horizontal connections in the developing visual cortex. *J. Neurosci.* **21**:3476–3482.

Tsuchida, T., M. Ensini, S.B. Morton et al. 1994. Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell* **79**:957–970.

Vaadia, E., I. Haalman, M. Abeles et al. 1995. Dynamics of neuronal interactions in monkey cortex in relation to behavioural events. *Nature* **373**:515–518.

von der Malsburg, C. 1981. The Correlation Theory of Brain Function. Tech. Rep. 81–2, Göttingen: Biophysical Chemistry, MPI.

Von Economo, C. 1929. The Cytoarchitectonics of the Human Cerebral Cortex. Oxford: Oxford Univ. Press.

White, E.L. 1989. Cortical Circuits: Synaptic Organization of the Cerebral Cortex. Boston: Birkhaüser.

Whittington, M.A., and R.D. Traub. 2003. Interneuron diversity series: Inhibitory interneurons and network oscillations *in vitro*. *TINS* **26**:676–682.

Woolsey, T.A., and H. Van der Loos. 1970. The structural organization of layer IV in the somatosensory region S1 of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectural units. *Brain Res.* **17**:205–242.

Yuste, R. 2005. Origin and classification of neocortical interneurons (short survey). *Neuron* **48**:524–527.

Yuste, R., J.N. MacLean, J. Smith, and A. Lansner. 2005. The cortex as a central pattern generator. *Nat. Rev. Neurosci*. **6**:477–483.

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